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The Journal of Allergy

VOL. 16 JULY, 1945 No. 4

American Academy of Allergy

First Annual Meeting, New York, N. Y., Dec. 11 and 12, 1944

(Continued)

THE TRANSPORT OF ANTIGEN THROUGH THE BODY BY ELECTROPHORESIS

ABRAHAM WALZER, M.D., AND HARRY G. GOLAN, M.D., BROOKLYN, N. Y.

GALVANIC current has long been in use as a means of introducing various substances into the skin and mucocutaneous tissues for the purpose of local therapy. The phenomena involving the electric transfer of percutaneously applied substances have been differentiated and variously designated as iontophoresis, cataphoresis, electroosmosis, and electrophoresis. Each of these terms has been employed to denote a different type of mechanism.

The present communication deals with the electric introduction and transport of antigen into the skin and through the human body. Since the mechanism involved in the transport of antigen through the body by an electric current is not clearly understood, the term electrophoresis was chosen as the one most suitable for the phenomenon. This term implies the introduction of a substance through the skin and its transport through the body by means of a galvanic current without specifying any particular mechanism by which this is accomplished.

Many substances have been introduced into the skin electrophoretically. They include simple medicaments such as copper, zinc, iodine, and calcium, as well as more complicated ones, such as morphine, cocaine, histamine, epinephrine, choline, mecholyl, and sulfanilamide. The percutaneous administration of protein antigen by electrophoresis has been reported previously by Abramson and his associates,[1] Dutton,[2] and Shilkret.[3] By means of the galvanic current, these workers introduced pollens, foods, and other allergens into the skin of hypersensitive patients and empoyed this method for the purpose of testing and treating them. The authors, on the other hand, have been primarily interested in the investigation of the electric transport of antigens through the body after their introduction into the skin by the same means. For these studies, it was found advantageous to use subjects who were not naturally sensitive to the antigens employed. In this manner, some of the difficulties encountered by previous workers were avoided.

From the Department of Dermatology and Syphilology and the Division of Allergy of the Jewish Hospital of Brooklyn.

Read at the First Annual Meeting of the American Academy of Allergy, New York City, Dec. 12, 1944.

I. TECHNIQUE

Normal (nonatopic) subjects were used in these studies. Cutaneous sites were passively sensitized in these subjects with reagin-bearing sera of high titer. These were obtained from sensitive patients who had responded with marked reactions to cutaneous tests with cottonseed, horse dander, ragweed, and peanut. A serum containing reagins for horse dander was employed in most of the experiments. About 0.05 ml. of undiluted serum was injected intracutaneously for local passive sensitization. Saline antigenic extracts, containing 0.1 mg. of total nitrogen per milliliter, were employed.

The apparatus employed in these experiments consisted of a simple battery of 22.5 volts with a milliammeter and a rheostat in the positive lead. Each electrode consisted of a pad composed of two round disks of blotting paper separated by a thin pledget of cotton. The disks were 3.84 cm. (1½ inches) in diameter, which was about 1.27 cm. larger than the metal electrodes and covered an area of about 11.6 square centimeters. The current density was 0.13 Ma. per square centimeter. The blotting paper disks were free of contaminating substances capable of producing wheals.

About forty-eight hours after local cutaneous sensitization, one paper pad, moistened with 2 ml. of antigen extract, was placed on a normal skin site and covered with the positive metal electrode. The other paper pad, moistened with 2 ml. of saline solution, was placed on the sensitized site and covered with the negative metal electrode. The electrodes were held in position on the extremities by elastic bands or, when the sites were on the trunk, by strips of adhesive tape. In most of the experiments, a current of 1.5 Ma. was employed for from fifteen to thirty minutes, although either the milliamperage or the time was occasionally varied. The other factors, however, such as the size of the wet electrodes, the quantity of the sensitizing serum, and the dilutions and amounts of the antigen were usually constant.

Using related serum and antigen, a wheal was usually obtained at the passively sensitized site, with the technique described, within thirty minutes. Nonrelated antigens did not induce whealing. The reaction, therefore, was specific. It was shown to be the result of an interaction between the fixed skin-sensitizing antibody at the negative pole and its related antigen electrically transported through the body from the point of its application at the positive pole (Fig. 1).

II. THE REACTIONS AT THE ELECTRODE SITES

After the current had been turned on, both pole sites on the skin were observed every five minutes. The first symptom occurred almost instantly and consisted of a tingling or pricking sensation at both pole areas, more pronounced, however, at the sensitized site or negative pole. Within five minutes, a diffuse or punctate erythema was usually noted at both areas, that at the sensitized or negative pole site being pronounced. These immediate reactions were undoubtedly due to the effects of the electric current and not to the transport of the antigen.

The erythema at the negative electrode or sensitized site rapidly became darker in color and gradually extended for about 1.3 cm. or more beyond the moist electrode. It soon was surrounded by a ring of erythematous points or spots. Within this erythematous area, whealing was usually noted about ten or fifteen minutes after the current was turned on. It began either as a central white or pinkish-white wheal or as a number of scattered, tiny wheals.

Occasionally, these fused into one wheal or, more frequently, several small wheals of various shapes and sizes, with or without pseudopods, developed. These wheals did not necessarily include the exact point where the sensitizing serum had been injected, but often formed near or around it.

Within fifteen to thirty minutes after turning on the current, the reaction reached the height of its development. At this time, it consisted of one or several wheals within a pronounced solid erythematous patch considerably larger than the moist electrode. Surrounding this patch of solid erythema, a halo of erythematous spots and points, about 2.5 to 3.8 cm. in diameter, was observed. This reaction simulated that previously described by Walzer and Walzer[4] in their study of wheal formation resulting at passively sensitized sites from stimulation by antigen enterally or parenterally introduced.

At the site of application of the antigen, covered with the positive pole, a pronounced patch of erythema developed which was sharply limited to the area under the moist electrode. This erythema varied in intensity in different subjects, but never was so extensive or so pronounced as that exhibited at the negative pole. No whealing occurred at the site covered with the positive-antigen pole.

The following is a detailed description of a single experiment.

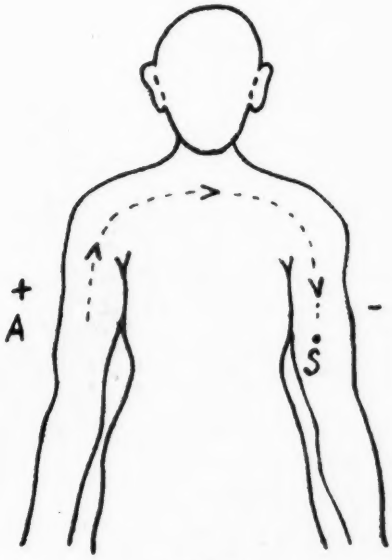

Fig. 1.

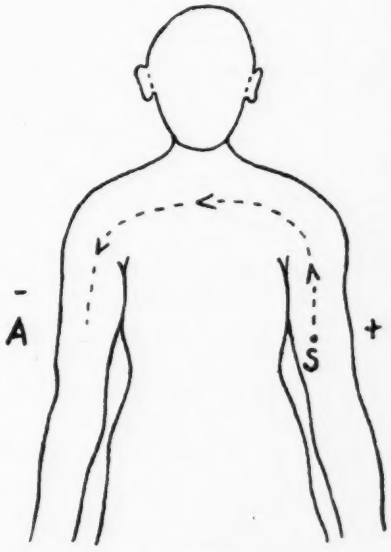

Fig. 2.

Fig. 1.—*A*, antigen; *S*, sensitized site; +, positive pole; and –, negative pole.
Fig. 2.—*A*, antigen; *S*, sensitized site; +, positive pole; and –, negative pole.

A cutaneous site on the left forearm of subject E. G. was passively sensitized with serum from a patient sensitive to horse dander. Forty-eight hours later, the sensitized site was covered with the negative-saline electrode. The positive-antigen pole was placed on a normal skin site on the opposite forearm. A current of 1.5 Ma. was employed. The reactions which developed at both terminals are chronologically described and compared in Table I.

After thirty minutes, the current was stopped and the electrodes were removed. The wheal on the left forearm then gradually began to regress and, in about forty-five minutes, had disappeared. A slight pruritus was noted at this time. The erythema at both sites persisted for a number of hours.

TABLE I

TIME (MIN.)	LEFT FOREARM SENSITIZED SITE, COVERED WITH THE NEGATIVE-SALINE POLE	RIGHT FOREARM NORMAL SITE, COVERED WITH THE POSITIVE-SALINE POLE
1	A faint tingling sensation was present	There was no change
5	A faint erythema was present	There was no change
10	A diffuse erythema was present	A slight punctate erythema, accompanied by a slight tingling sensation, was present
15	The erythema had increased in extent and intensity, and a small, slightly raised, pinkish wheal had formed in the center. The tingling sensation was still present	There was only a slight increase in the erythema. Wheal formation was completely absent
20	The erythema was more intense and extended about 1.3 cm. beyond the margin of the moist electrode. The wheal was larger, more elevated and indurated, and showed pseudopods at the upper border. A collar of scattered erythematous points and spots, about 1.3 cm. wide, had formed around the whole reaction. A tingling sensation was still present.	There was no further change in the reaction
25	The erythema was slightly more intense. The wheal remained unchanged. The erythematous scattered halo had increased slightly	The reaction remained unchanged
30	The erythema and its halo remained unchanged. The wheal, although slightly larger, was less indurated	The erythema remained unchanged, and wheal formation was absent

III. THE EFFECT OF REVERSING THE DIRECTION OF THE CURRENT, WITH THE ANTIGEN AND THE SALINE IN THE SAME POSITION

Wheal reactions were obtained at will with the above technique. In one series of experiments, the routine technique was changed, and the positions of the metal electrodes were reversed. The sensitized site was made the saline-positive pole and the normal site the antigen-negative pole. The current, therefore, flowed from the sensitized site to the antigen. With the same amount of current employed in the previous experiments, no reaction occurred at the sensitized site within forty-five minutes (Fig. 2). In this manner, it was demonstrated that the transport of antigen through the body was successful only when it was applied at the positive pole, with the current flowing toward the sensitized site.

IV. MULTIPLE SENSITIZED SITES COVERED WITH ELECTRODES FROM A COMMON LEAD

In these experiments, three sensitized sites on a subject were covered with saline electrodes, all leading from a single negative pole. The antigen was applied at the positive pole at a distant normal site. With a current of 1.5 Ma., reactions occurred at each of the sensitized sites within thirty minutes (Fig. 3). This demonstrated that several sensitized sites on the same subject could react to related transported antigen if the sites were covered with negative-saline electrodes from a common lead.

V. THE EFFECT OF APPLYING THE ANTIGEN DIRECTLY OVER THE SENSITIZED SITE

In these experiments, the antigen was placed over the sensitized site and the saline over a normal site. With this arrangement, a reaction developed at the sensitized site when covered by either pole. It appeared rapidly and was completed in about fifteen minutes. However, the positive pole over the sensitized site induced a larger wheal than the negative pole in the same position (Fig. 4).

These results, obtained with passively sensitized sites on nonsensitive subjects, are similar to those elicited by Abramson and his associates,[1] by Dutton,[2] and by Shilkret,[3] all of whom worked on hypersensitive patients. They obtained marked wheal reactions when using either the positive or negative poles for the direct electric introduction of pollen, food, and other antigens into the skin. Hence, it would appear that either pole may be utilized to introduce antigens directly into the skin by electrophoresis, but that the transport of antigen electrophoretically through the body can only be accomplished by its application at the positive pole.

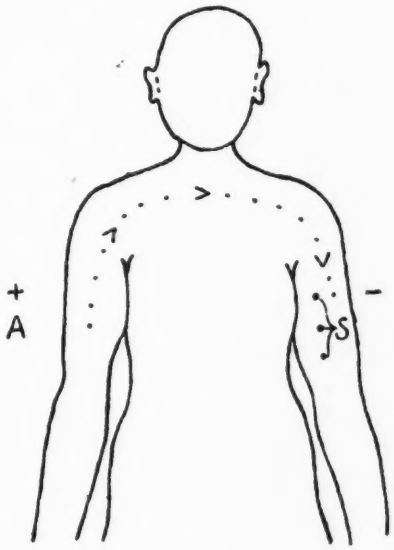

Fig. 3.

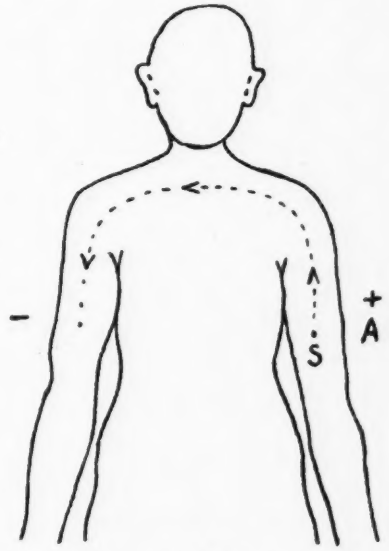

Fig. 4.

Fig. 3.—*A*, antigen; *S*, sensitized sites; +, positive pole; and –, negative pole.
Fig. 4.—*A*, antigen; *S*, sensitized site; +, positive pole; and –, negative pole.

VI. THE EFFECT OF ELECTRICALLY TRANSPORTED RELATED ANTIGEN ON SENSITIZED SITES IN THE PATH OF THE ELECTRIC CURRENT

The object of these experiments was to determine whether antigen which was being transported to a sensitized area by electrophoresis, would influence other similarly sensitized sites in its path. These intervening sensitized sites were not covered with electrodes.

Two sites, about 8 cm. apart in the vertical axis on the arm were similarly sensitized. About forty-eight hours later, the negative-saline electrode was applied to the lower sensitized site and the positive-antigen electrode was placed on a normal skin site on the other arm. This left the upper sensitized site uncovered by an electrode but in the path of the electric current running between the two electrodes. With a current of 1.5 Ma., a reaction occurred on the sensitized site covered with the negative-saline electrode in twenty minutes.

At forty-five minutes, no change had occurred at the exposed sensitized site (Fig. 5).

These experiments were repeated with the sensitized sites placed about 8 cm. apart horizontally on the arm. The negative-saline electrode was applied to the outer sensitized site and the positive-antigen electrode to a normal site on the other arm. The results obtained were similar to those described in the previous experiment. The significance of these findings will be discussed subsequently.

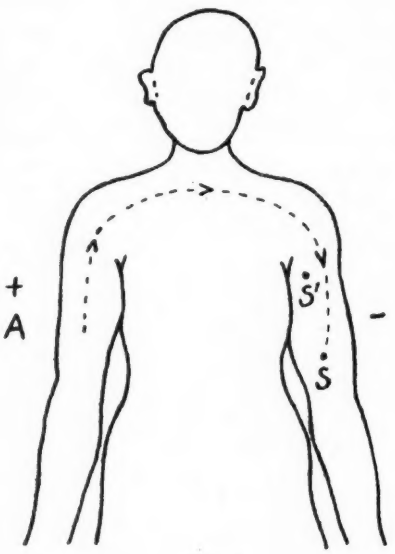

Fig. 5.

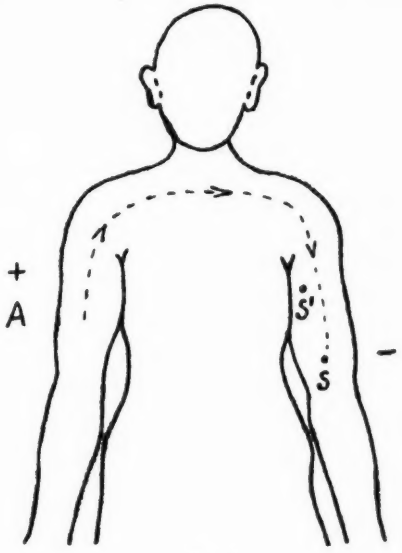

Fig. 6

Fig. 5.—*A*, antigen; *S*, sensitized site covered with negative electrode; *S'*, sensitized site exposed; +, positive pole; and −, negative pole.

Fig. 6.—*A*, antigen; *S*, sensitized site covered with negative electrode; *S'*, sensitized site traumatized by heat or friction; +, positive pole; and −, negative pole.

VII. THE EFFECT OF TRAUMA ON SENSITIZED SITES IN THE PATH OF RELATED ELECTRICALLY TRANSPORTED ANTIGEN

In previous studies on whealing by Walzer and Walzer,[4] it was noted that mechanical stimuli, such as rubbing or brushing, when applied to a sensitized site before the onset of a reaction, accelerated the appearance and development of the wheal. In those experiments, the antigen was present in the circulation.

With these results in mind, experiments were carried out to determine whether a sensitized site, in the path of an electric current transporting related antigen, could be induced to react by traumatizing the site with various physical agents.

A. Trauma by the Application of Heat.—Two sites on one arm, about 8 cm. apart in the vertical direction, were sensitized as in a previous experiment. The negative-saline electrode was applied to the lower site and the positive-antigen electrode was placed on a normal area on the other arm. A current of 1.5 Ma. was employed. During the flow of current a test tube containing water at 46° C. was applied constantly to the interposed sensitized site. A wheal developed only at the lower sensitized site covered by the negative pole. The only change noted at the interposed site was a slight erythema produced by the heat.

B. Trauma by Friction.—The above experiment was repeated using friction, instead of heat, as the traumatic factor. The friction agent, a brush, was applied

to the interposed sensitized site during the flow of the current. As in the previous experiment, no reaction occurred at the traumatized site, except an erythema resulting from the friction. At the negative pole a typical wheal reaction occurred (Fig. 6).

All of these experiments indicate that the antigen is electrically charged and moves under the influence of the electric current in a direct line from the positive to the negative pole. Only at this latter point does it lose its charge and become free unelectrified antigen, capable of uniting with its related antibodies.

It is evident, therefore, that no antigen escapes into the circulation during its passage from the positive to the negative pole, since only those sensitized sites covered with the negative pole light up. Stimulation, by heat or friction, of uncovered sites in the path of the transported antigen did not induce whealing as these stimuli could not release any antigen from its electric charge.

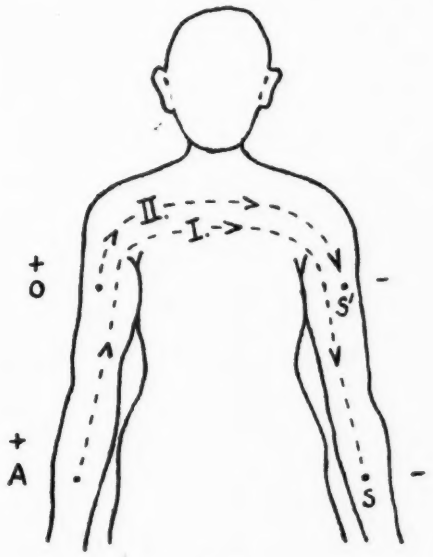

Fig. 7.

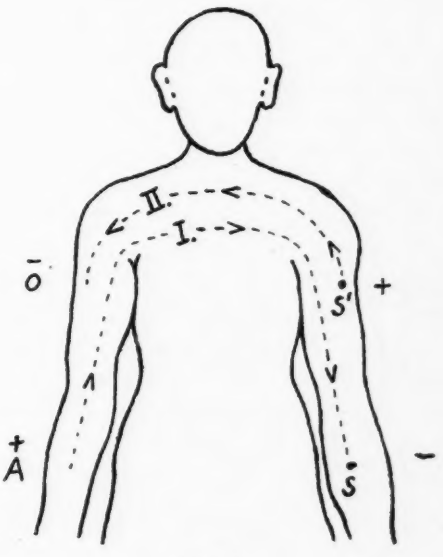

Fig. 8.

Fig. 7.—Circuit *I*: (Lower) *A*, antigen; and *S*, sensitized site. Circuit *II*: (Upper) *O*, saline; *S'*, sensitized site; +, positive pole; and −, negative pole.

Fig. 8.—Circuit I: *A*, antigen; and *S*, sensitized site. Circuit II: *O*, saline; *S'*, sensitized site; +, positive pole; and −, negative pole.

VIII. THE EFFECT OF ELECTRICALLY TRANSPORTED ANTIGEN OF ONE CURRENT UPON A SENSITIZED SITE IN A NONANTIGEN-BEARING CIRCUIT

The purpose of these experiments was to determine whether electrically transported antigen of one circuit (Circuit I) would induce a reaction at a sensitized site in a second circuit which was not carrying antigen (Circuit II).

A. When Circuit II Lies Within the Path of Circuit I, Both Running in the Same Direction Toward the Sensitized Sites.—Two sites on the same extremity, one on the arm and the other on the forearm, were sensitized with the same serum. The forearm site was covered with the saline-negative pole of Circuit *I* and the antigen-positive pole of this circuit was placed on a normal site on the opposite forearm (Fig. 7). The saline-negative pole from another galvanic battery unit (Circuit *II*) was applied to the sensitized site on the arm and the saline-positive pole was applied to a normal area on the opposite arm. Hence,

there were two separate circuits, with currents running in the same direction, i.e., toward the sensitized sites. Antigen was present only in the longer circuit connecting the forearms. The circuits, each carrying 1.5 Ma., were closed simultaneously. In fifteen minutes, reactions appeared at both sensitized sites, despite the fact that no antigen was carried by Circuit *II*. The wheal reaction on the arm (Circuit *II*) was larger than that on the forearm (Circuit *I*), despite the fact that the antigen producing both reactions was introduced only at the positive pole of Circuit *I*.

These experiments were repeated with the current of the antigen-bearing circuit (Circuit *I*) increased to 3 Ma., or double that of Circuit II. Under these conditions the wheal at the sensitized site of Circuit *I* on the forearm was larger than that of Circuit *II* on the arm.

B. When Circuit II Lies Within the Path of Circuit I, Both Running in Opposite Directions.—The object of these experiments was to determine whether the sensitized site of Circuit *II* would react with the antigen in Circuit *I*, when the currents ran in opposite directions. Two sites were sensitized on the same extremity as in the previous experiments (Fig. 8). Antigen-bearing Circuit *I*, connecting the forearms, was in the same position as in the previous experiments, i.e., the negative-saline electrode was over the sensitized site on one forearm and the positive-antigen electrode was on the other. In Circuit *II* connecting the arms, the positive-saline electrode was placed over the sensitized site on the arm and the negative-saline electrode was on the opposite arm. Hence, in the forearm circuit (*I*), the current carrying antigen traveled toward the sensitized site. In the arm circuit (*II*), the current traveled within the path of Circuit *I* but in the opposite direction, i.e., away from the sensitized site. With both currents equal and flowing simultaneously, a reaction occurred only at the sensitized site on the forearm in Circuit *I*.

Similar experiments were carried out with the amount of current in the arm circuit (II) doubled. The results were the same. A reaction occurred only at the sensitized site of the forearm in Circuit I.

C. When Circuit I and Circuit II Cross Each Other.—The purpose of these experiments was to determine whether the sensitized site of Circuit II would react if Circuit I and Circuit II crossed.

Two sites were sensitized on the back of a subject, one near the base of the neck and the other below the left scapula. Circuit *I*, carrying the antigen, ran vertically up the back with the negative-saline pole on the sensitized site at the base of the neck and the positive-antigen pole applied in the lumbar region (Fig. 9). Circuit *II* ran transversely across the back with saline at both poles, the negative pole on the sensitized site below the left scapula, and the positive pole below the right scapula. The currents of both circuits were turned on at the same time, each carrying 1.5 milliamperes. Within ten minutes, whealing had begun at the sensitized site in the vertical or antigen-bearing circuit (*I*). In fifteen minutes, whealing was noted at the sensitized site in Circuit *II*. In twenty minutes, both wheals were well formed, the one at the base of the neck (Circuit *I*) being more pronounced than that below the left scapula (Circuit *II*).

This experiment was repeated several times with the circuits crossing at angles up to 135 degrees. Reactions occurred at about the same time and to the same degree as in the original experiments when the circuits crossed each other at right angles.

In the first of the above experiments, Circuit II flows within the same electric path and in the same direction as antigen-bearing Circuit I. The sensitized site of Circuit II is, therefore, within the path of the positively charged antigen carried by Circuit I. As the antigen passes this site at the negative pole of Circuit II, some of it is deprived of its charge and combines with the antibodies to induce a reaction.

In the experiments in which the direction of the current of Circuit II is reversed, the sensitized site is at the positive pole of that circuit. Since the antigen in Circuit I is also positively charged, it is not deprived of its charge as it passes the sensitized site of Circuit II. Hence no reaction occurs.

In the experiments in which the Circuits I and II cross, some of the antigen of Circuit I is apparently deflected, at the point of crossing, toward the negative pole and sensitized site of Circuit II. Here it is deprived of its charge and reacts with the local antibodies to form a wheal.

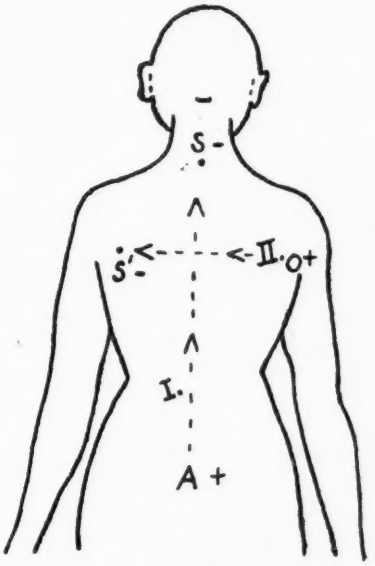

Fig. 9.

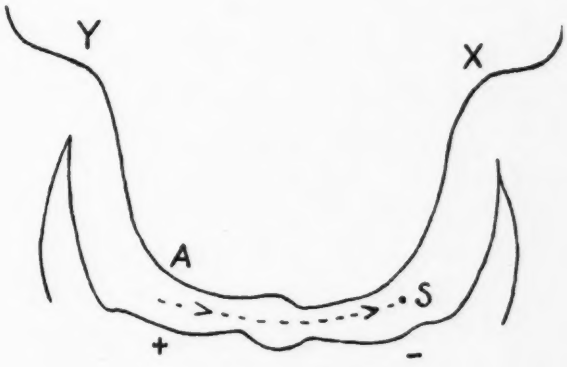

Fig. 10.

Fig. 9.—Circuit I: *A*, antigen; and *S*, sensitizing site. Circuit II: *O*, saline; *S'*, sensitizing site; +, positive electrode; and –, negative electrode.

Fig. 10.—*X*, subject with sensitized site; *Y*, subject receiving antigen; *A*, antigen; *S*, sensitized site; +, positive pole; and –, negative pole.

IX. TRANSPORT OF ANTIGEN FROM ONE SUBJECT TO ANOTHER

These experiments were performed to determine whether antigen could be transported by an electric current through individuals in contact with each other and within a single electric circuit.

A. The Transport of Antigen From the Upper Extremity of One Subject to the Upper Extremity of Another Through Clasped Hands.—A site on the right forearm of Subject *X* was passively sensitized. About forty-eight hours later, the right hand of Subject *X* was covered with gauze moistened with tap water and clasped by the right hand of Subject *Y*. Both hands were then tightly bandaged. The negative-saline pole was applied to the sensitized site on the right forearm of Subject *X* and the positive-antigen pole was placed on the right forearm of Subject *Y* (Fig. 10). With a current of 1.5 Ma., a wheal developed at the sensitized site on the forearm of Subject *X* in fifteen minutes.

The experiment was repeated, the sensitized site and the poles being placed on the arms. The results were the same. Hence, the antigen had been trans-

ported from the upper extremity of Subject *Y* through the hands to the upper extremity of Subject *X*.

B. The Transport of Antigen Through the Bodies of Two Subjects.—A site on the left arm of Subject *X* was passively sensitized. About forty-eight hours later, the right arm of Subject *X* was wrapped in gauze, soaked in tap water, and snugly bandaged to the left arm of Subject *Y*. The negative-saline electrode was placed over the sensitized site of the left arm of Subject *X*, and the positive-antigen electrode was applied to the right arm of Subject *Y*. A current of 2 Ma. was employed. Within thirty minutes, a wheal developed at the sensitized site of Subject *X*. Hence, the antigen had been transported through the bodies of both subjects from the right arm of Subject *Y* to the left arm of Subject *X*.

This experiment was repeated with the subjects in contact through clasped right hands. The negative-saline electrode was placed on a sensitized site on the left arm of Subject *X* and the positive-antigen electrode on the left arm of Subject *Y*. The reaction occurred in about fifteen minutes. The antigen had passed through the bodies of both subjects by way of clasped hands.

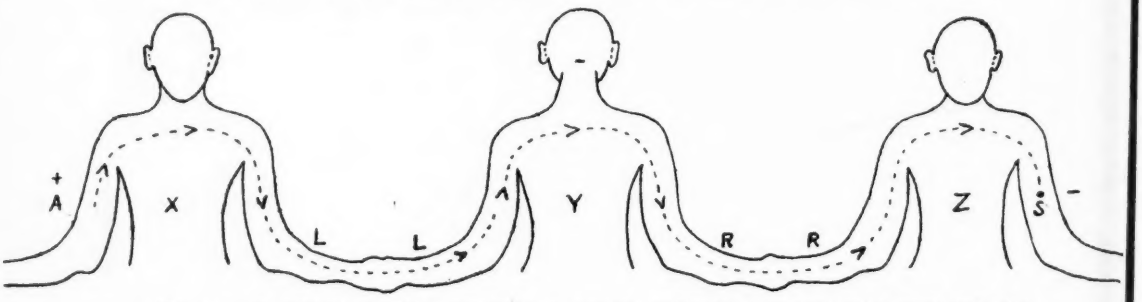

Fig. 11.—*X*, subject receiving antigen; *Y*, neutral subject; *Z*, subject with sensitized site; *A*, antigen; *S*, sensitized site; +, positive pole; and −, negative pole; *L*, left upper extremity; *R*, right upper extremity.

C. The Transport of Antigen Through the Bodies of Three Subjects.—Contact between the three subjects was made in the following manner: The left hand of Subject *X* clasped the left hand of Subject *Y* whose right hand clasped the right hand of Subject *Z*. The sensitized site, covered with the negative-saline electrode, was on the left arm of Subject *Z* and the positive-antigen electrode on the right arm of Subject *X* (Fig. 11). The antigen, therefore, had to pass through the upper extremities and trunks of three persons to reach the sensitized site. A current of 5 Ma. was employed. A very pronounced wheal reaction developed at the passively sensitized site in twenty-five minutes.

These experiments are of interest, not only because of the distance to which the antigen was electrically transported, but also because they demonstrate that this antigen is not transported by the circulation but follows the course of the electric current, moving in a direct path from the positive to the negative pole. They also show that the skin is probably not so resistant to the penetration of cutaneously applied substances as is generally believed. The electrically charged antigen penetrated a series of five skin sites in a relatively short time with the use of only a weak current.

CONCLUSIONS

1. Horse dander, cottonseed, ragweed, and peanut antigens were not only introduced into the skin by electrophoresis, but were also transported through the body by the same means.

2. By electrophoresis, the antigens were transported through three subjects in the same circuit.

3. Although the antigens may be introduced into the skin by either positive or negative pole, they could be transported through the body to a distant site only by the application with the positive pole.

4. Any number of sensitized sites reacted to transported antigen when they were covered with negative-saline poles from a common lead.

5. Sensitized sites lying in the path of electrically transported antigen did not react except when they were covered with a negative pole of the same or another circuit. Mechanical stimulation of these sensitized sites did not alter the result.

6. A sensitized site covered with a negative pole of a nonantigen-bearing circuit, reacted with antigen of another circuit when the two circuits ran parallel or crossed each other.

7. Electrically transported antigen was not present in the circulation but moved directly in an electrically charged state along the path of the electric current from the positive to the negative pole.

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Original Articles

THE IMMUNOCHEMISTRY OF ALLERGENS

VII. A Study of the Homogeneity of Cottonseed-Globulin Preparations by Anaphylactic Reactions*†

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ISOLATION of a single allergenic component of cottonseed in the proteic-polysaccharidic fraction, CS-1A, led to detection of another cottonseed allergen.[1] This second allergen was contained in the globulin fraction. It was recognized, and differentiated from CS-1A, by cutaneous reactions of passively sensitized subjects.

Separation of CS-1A from other antigens of the cottonseed depended on the stability of this protein component which resisted denaturation and inactivation when subjected to rigorous fractionation and purification procedures.

CS-1A differs widely from the cottonseed globulins in solubility. Nevertheless, the globulin preparations, when purified by repeated solution and precipitation, retained the water-soluble CS-1A in quantities sufficient to induce anaphylactic shock in guinea pigs previously sensitized with this antigen.[1] It was then recognized that the specific allergenic activity of the purified globulin fractions might be due to a water-soluble contaminant, other than CS-1A, that was not separable by repeated solution and precipitation of the globulins. This tendency of precipitated globulins to carry other proteins with them, by chemical union or adsorption, was encountered by Hektoen and Cole[2] in their study of globulins of eggs and serum.

This report presents observations which demonstrate that the cottonseed-globulin preparations were contaminated with a water-soluble antigen in addition to, and differentiable from, CS-1A. Whether the specific allergenic activity of the cottonseed globulins is attributable to the unidentified water-soluble contaminant alone has not been determined.

FRACTION-DESIGNATION AND PREPARATION OF TEST SOLUTIONS

CSG. Cottonseed Globulin.—The method of preparation of this globulin fraction, previously described in detail as globulin 2CS-1[1] is here summarized. Defatted cottonseed was extracted ten times with distilled water, and the extracts were discarded. The residue was then extracted with 0.5 N sodium chloride solution. The globulins were precipitated from this extract by dilution with water. The precipitated globulins were redissolved in saline solution. This process was repeated nine times. The final solution contained 1.182 mg. of nitrogen per milliliter.

CSG-H. Heated Cottonseed Globulin.—This preparation was identical with fraction 2CS-2 described previously.[1] A portion of CSG was heated at 83° to 85° C. for one hour. After cooling, the coagulum was removed and discarded.

*Contribution from Allergen Investigations, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

†For Paper VI of this series see Reference 7.

The heated globulin was purified by alternately precipitating in distilled water and redissolving in 0.5 N saline solution, to a total of five precipitations. The final solution contained 0.498 mg. of nitrogen per milliliter.

UCS. Unfractionated Water-Extract of Cottonseed.—Five grams of defatted cottonseed were extracted overnight with 95 ml. of distilled water saturated with toluene. The residue was separated by centrifugation and the supernate was Seitz-filtered under pressure of carbon dioxide. The extract contained 1.02 mg. of nitrogen per milliliter, of which 58 per cent were precipitable by phosphotungstic acid.

UCS(S). Unfractionated Salt Extract of Cottonseed.—A 1:20 salt extract was prepared as described in the preceding paragraph, except that 0.5 N sodium chloride was the extracting medium. The salt extract, UCS(S), contained 3.24 mg. of nitrogen per milliliter, of which 79.4 per cent were precipitable by phosphotungstic acid.

CS-1A. Proteic-Polysaccharidic Fraction.—The procedure used to isolate CS-1A from cottonseed has been described elsewhere.[3] CS-1A, which represented about 1 per cent of the defatted seed, contained 11.8 per cent nitrogen and 39.9 per cent carbohydrate.

CS-51R. Allergenic Proteic Fraction Isolated From CS-1A.—CS-51R represents the principal proteic component of CS-1A.[4] This fraction contained 19.8 per cent nitrogen and 0.9 per cent carbohydrate.

EXPERIMENTAL

Detection of CS-1A in CSG and CSG-H by Gross Anaphylaxis.—For detection of CS-1A by gross anaphylaxis, ten guinea pigs were sensitized with CS-1A. Each animal received a single intra-abdominal injection containing 5 mg. of CS-1A in 1 ml. of saline. Three weeks later each animal received a single intravenous injection of one of the globulin preparations. Each challenging injection contained 1 mg. of protein nitrogen (P.N.). Five animals were tested for sensitivity to CSG and five to CSG-H. The results are shown in Table I. When injected with CSG, two of the five animals previously sensitized with CS-1A died from immediate anaphylactic shock; three animals reacted with moderate to severe symptoms but recovered. One control animal exhibited no symptoms. Of the five animals injected with the CSG-H preparation, one succumbed to fatal anaphylactic shock; the other four reacted with slight to severe symptoms and

TABLE I. DETECTION OF CS-1A IN GLOBULIN PREPARATIONS BY GROSS ANAPHYLAXIS IN GUINEA PIGS SENSITIZED TO CS-1A*

ANIMAL	RESULTS OF INTRAVENOUS INJECTION OF GLOBULIN PREPARATIONS CONTAINING 1 MG. OF PROTEIN NITROGEN
	CSG (unheated globulin)
3620	Fatal anaphylaxis, three and one-half minutes
3633	Fatal anaphylaxis, five minutes
3657	Moderate anaphylaxis, with recovery
3664	Moderate anaphylaxis, with recovery
3670	Severe anaphylaxis, with recovery
3663†	None
	CSG-H (heated globulin)
3570	Severe anaphylaxis, with recovery
3615	Slight anaphylaxis, with recovery
3618	Fatal anaphylaxis, three minutes
3625	Moderate anaphylaxis, with recovery
3632	Severe anaphylaxis, with recovery

*Body weight, when sensitized, ranged from 297 to 310 grams. Each animal was sensitized with 5 mg. of CS-1A. Incubation period was three weeks.

†Normal control, not sensitized.

recovered. Comparison of these results with the previously recorded quantitative studies[5] of the shocking capacity of CS-1A in similarly sensitized animals indicates that the globulin preparations contained, on a nitrogen basis, about 0.2 to 0.3 per cent of CS-1A.

Detection of CS-1A in CSG and CSG-H by the Schultz-Dale Method.— Schultz-Dale tests were conducted with uterine muscles from sensitized guinea pigs as described in previous communications from this laboratory.[6, 7] Guinea pigs were sensitized either by subcutaneous injection of alum-treated solutions or by intra-abdominal injections of untreated solutions. The method of sensitization and quantity of preparations used are indicated for each animal both in the tables and in the figure recording the results. When contraction of the uterine muscle resulted from addition of a preparation, the bath was flushed with fresh Tyrode's solution, and the same preparation was added again to test for desensitization before addition of the next preparation. To save space, additions of antigen to prove desensitization are not recorded in the tables.

The relationship of CS-1A to CSG-H was investigated by the cross-reactions recorded in Table II. Uterine muscle from each of four animals previously sensitized to CS-1A was tested for sensitivity to CSG-H. No reactions were induced in the uterine muscles from two of these animals (3667 and 3915) when 10,000 protein nitrogen units (PNU)* of CSG-H were added to the bath. Positive reactions occurred however in two cases (Animals 3680 and 3917) when the first addition of CSG-H was increased to 100,000 PNU, and these reactions desensitized the tissues to subsequent test with 100 PNU of CS-1A and 10 PNU of CS-51R, respectively. When the second uterine horns from these same animals were first desensitized to 100 PNU of CS-1A or to 10 PNU of CS-51R, the tissues did not contract when 100,000 PNU of CSG-H were added.

TABLE II. DETECTION OF CS-1A IN CSG-H BY THE DALE METHOD

ANIMAL	SENSITIZING ANTIGEN	INCUBATION PERIOD (DAYS)	HORN NO.	PREPARATION ADDED	PROTEIN NITROGEN UNITS	UTERINE CONTRACTIONS
3667	CS-1A 0.5 mg. P.N. i.a.*	21	1	CSG-H	10,000	0
				CS-1A	1,000	++++
				Histamine		++++
			2	CS-1A	1,000	++++
				Histamine		++++
3915	CS-1A 0.5 mg. P.N. Alum precipitated s.c.†	30	1	CSG-H	10,000	0
				CS-51R	100	++++
				Histamine		++++
			2	CS-51R	100	++++
				CSG-H	100,000	0
				Histamine		+++
3680	CS-1A 0.5 mg. P.N. i.a.	21	1	CSG-H	100,000	+++
				CS-1A	100	0
				CS-1A	1,000	+
				Histamine		++++
			2	CS-1A	100	++++
				CSG-H	100,000	0
				CS-1A	1,000	+
				Histamine		++++
3917	CS-1A 0.5 mg. P.N. Alum precipitated s.c.	33	1	CSG-H	100,000	++++
				CS-51R	10	0
				CS-51R	100	+++
				Histamine		++++
			2	CS-51R	10	++++
				CSG-H	100,000	0
				Histamine		++++

*Sensitized by intra-abdominal injection.
†Sensitized by subcutaneous injection.

*One protein nitrogen unit is equivalent to 0.00001 mg. of phosphotungstic-acid-precipitable nitrogen.

These results showed that CSG-H contained a small but detectable quantity of CS-1A and confirmed the results of the gross anaphylaxis experiments.

Records of uterine contractions presented in Table III show that sensitivity to CS-1A is not readily induced in guinea pigs by injection of CSG or CSG-H, even though the same dosages of these fractions were sufficient both to elicit contractions in uterine muscles sensitized to CS-1A and to shock guinea pigs previously sensitized to CS-1A. Uterine muscles from two animals (4020 and 4032) previously sensitized with dosages equivalent to 0.5 mg. of protein nitrogen from CSG and CSG-H, respectively, gave no response to 10,000 PNU of CS-1A. Sensitizing doses of CSG equivalent to 1 mg. protein nitrogen induced slight sensitivity to CS-1A in one animal (3738) and no sensitivity to CS-1A in another (3736). Each of these four animals developed antibodies for the sensitizing antigen.

Reactions of the uterine muscle from Animal 3736 were especially significant. This animal was sensitized with CSG. CS-1A induced no contraction in uterine horn No. 1 from this animal. The muscle was then stimulated by, and desensitized to, CSG. A subsequent test of the same muscle with UCS induced a slight contraction. This reaction suggested that CSG sensitized to some water-soluble component of cottonseed distinct from CS-1A. Investigation of this possibility by cross-reactions yielded the results recorded in Table IV. Typical of these results are the reactions of the uterine muscle from Animal 3684. This animal was sensitized to CSG. Uterine horn No. 1 was desensitized with CSG, and was then shown to be insensitive to CS-1A. A subsequent addition of UCS, however, induced a maximal contraction. Uterine horn No. 2 from the same animal gave an initial submaximal contraction to CS-1A, and subsequently reacted to CSG. But these reactions to CS-1A and to CSG failed to neutralize the sensitivity to UCS. Similar results were obtained with the uterus of Animal 3671, which had been sensitized to CSG-H, excepting that sensitivity to CS-1A did not develop.

TABLE III. ANTIGENIC RELATIONSHIP OF CSG AND CSG-H TO CS-1A

ANIMAL	SENSITIZING ANTIGEN	INCUBATION PERIOD (DAYS)	HORN NO.	PREPARATION ADDED	PROTEIN NITROGEN UNITS	UTERINE CONTRACTIONS
4020	CSG 0.5 mg. P.N. i.a.	21	1	CS-1A	10,000	0
				CSG	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++
			2	CSG	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++
4032	CSG-H 0.5 mg. P.N. i.a.	26	1	CS-1A	10,000	0
				CSG-H	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++
			2	CSG-H	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++
3736	CSG 1.0 mg. P.N. i.a.	28	1	CS-1A	10,000	0
				CSG	10,000	++++
				UCS	10,000	+
				Histamine	10,000	++++
			2	CSG	10,000	++++
				UCS	10,000	±
				Histamine		++++
3738	CSG 1.0 mg. P.N. i.a.	28	1	CS-1A	10,000	+
				UCS	10,000	++++
				CSG	10,000	++++
			2	CSG	10,000	++++
				UCS	10,000	0
				Histamine		++++

TABLE IV. ANTIGENIC RELATIONSHIP OF UCS TO CSG AND CSG-H

ANIMAL	SENSITIZING ANTIGEN	INCUBATION PERIOD (DAYS)	HORN NO.	PREPARATION ADDED	PROTEIN NITROGEN UNITS	UTERINE CONTRACTIONS
3678	CSG 1.0 mg. P.N. i.a.	23	1	CSG	10,000	++++
				UCS	10,000	+++
				Histamine		++++
			2	UCS	10,000	++++
				CSG	10,000	+++
				Histamine		++++
3684	CSG 1.0 mg. P.N. i.a.	28	1	CSG	10,000	++++
				CS-1A	10,000	0
				UCS	10,000	++++
			2	CS-1A	10,000	+++
				CSG	10,000	++++
				UCS	10,000	+++
				Histamine		++++
3665	CSG-H 1.0 mg. P.N. i.a.	21	1	CSG-H	10,000	++++
				UCS	10,000	++++
			2	UCS	10,000	++++
				CSG-H	10,000	+++
				Histamine		++++
3671	CSG-H 1.0 mg. P.N. i.a.	23	1	CSG-H	10,000	++++
				UCS	10,000	++++
			2	CS-1A	10,000	0
				CSG-H	10,000	++++
				UCS	10,000	++++
3786	UCS 1.0 mg. P.N. i.a.	23	1	CSG	10,000	++++
				UCS	10,000	++++
			2	UCS	10,000	++++
				CSG	10,000	0
				Histamine		++++
3809	UCS 1.0 mg. P.N. i.a.	25	1	CSG	10,000	0
				UCS	10,000	++++
3789	UCS 1.0 mg. P.N. i.a.	24	1	CSG-H	10,000	++
				UCS	10,000	++++
			2	UCS	10,000	++++
				CSG-H	10,000	0
				Histamine		++++
3805	UCS 1.0 mg. P.N. i.a.	24	1	CSG	10,000	±
				CSG-H	10,000	0
				UCS	10,000	++++
			2	CSG-H	10,000	0
				CSG	10,000	0
				UCS	10,000	++++

Animals sensitized with the aqueous extract of cottonseed, UCS, usually developed only slight or no sensitivity to the globulin preparation (see last four experiments in Table IV); and those muscles that exhibited sensitivity to the globulin preparations on first addition were completely desensitized to CSG or CSG-H by a preceding reaction with UCS. On the other hand, uterine muscle from animals sensitized to CSG or CSG-H was not desensitized to CSG or CSG-H, respectively, by reaction with UCS (Animals 3678 and 3665).

The foregoing observations indicate that the purified globulin preparations retained a water-soluble, antigenic protein in addition to, and distinct from, CS-1A. This protein was present in both CSG and in CSG-H in concentration sufficient to sensitize the uterine muscle, but insufficient to desensitize the tissues to the greater concentration of the same antigen that was present in the water-extract, UCS. Thus the unidentified water-soluble antigen exhibited the usual quantitative relationship between dosages required to sensitize and to induce shock, whereas by contrast, a larger dose of CS-1A is required to sensitize than to shock.[5]

Results recorded in Table V are presented to show that the salt extract of

cottonseed, UCS(S), which contains a high proportion of globulin, completely desensitized uterine muscles from animals previously sensitized to CSG or CSG-H.

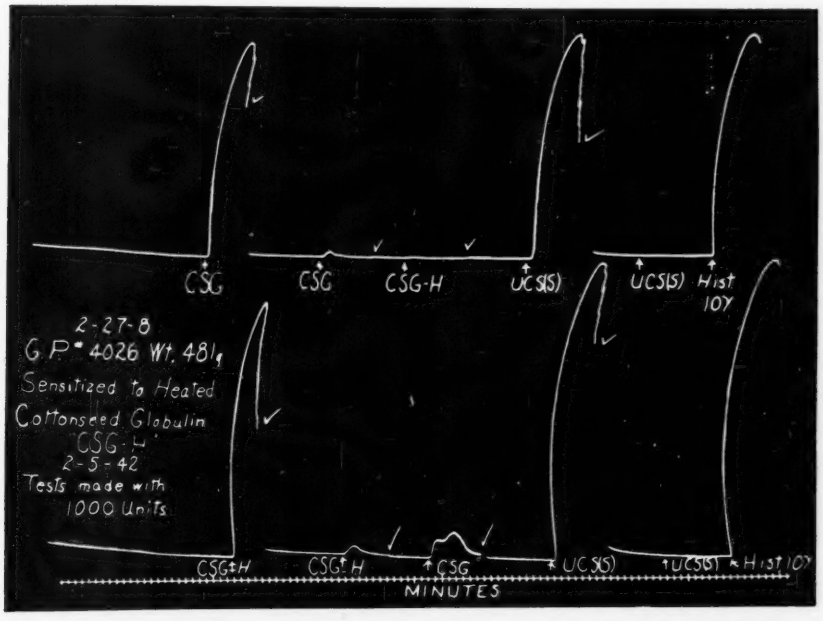

Fig. 1.—Antigenic relationship of CSG-H to CSG. This experiment also shows that CSG-H contained another antigen of whole cottonseed extract in concentration sufficient to sensitize, but neither CSG nor CSG-H contained this antigen in concentration sufficient to desensitize the tissue to UCS(S).

TABLE V. ANTIGENIC RELATIONSHIP OF UCS(S) TO CSG AND CSG-H

ANIMAL	SENSITIZING ANTIGEN	INCUBATION PERIOD (DAYS)	HORN NO.	PREPARATION ADDED	PROTEIN NITROGEN UNITS	UTERINE CONTRACTIONS
3976	CSG 1.0 mg. P.N. i.a.	28	1	UCS(S)	1,000	++++
				CSG	1,000	0
				Histamine		++++
			2	CS-1A	10,000	0
				CSG	1,000	+++
				Histamine		++++
3975	CSG 1.0 mg. P.N. i.a.	27	1	UCS(S)	10,000	++++
				CSG	1,000	0
				Histamine		++++
			2	UCS(S)	10,000	++++
				CSG	10,000	0
				Histamine		++++
3981	CSG 1.0 mg. P.N. i.a.	29	1	UCS(S)	1,000	++++
				CSG	1,000	0
				Histamine		++++
4017	CSG 0.5 mg. P.N. i.a.	20	1	UCS(S)	1,000	++++
				CSG	1,000	0
				Histamine		++++
			2	CSG	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++
4025	CSG-H 0.5 mg. P.N. i.a.	21	1	UCS(S)	1,000	++++
				CSG-H	1,000	0
				Histamine		++++
			2	CSG-H	1,000	++++
				UCS(S)	1,000	0
				Histamine		++++

ANTIGENIC RELATIONSHIP OF CSG AND CSG-H

The antigenic relationship of the unheated cottonseed globulin, CSG, to the heated globulin, CSG-H, is shown in Table VI. The results are briefly summarized: (a) When animals were sensitized to CSG, preparation CSG-H induced contractions in the uterine muscles but did not desensitize to CSG. (b) When animals were sensitized with CSG-H, preparation CSG-H desensitized to CSG. (c) CSG always desensitized to CSG-H.

The cross-reactions of CSG-H with CSG (Table VI) and with UCS(S) (Table V) show that the specificity of the protein antigens contained in CSG-H was identified with antigens contained in the unfractionated salt extract and in the unheated globulin preparation. Thus, the specificity of the protein contained in CSG-H was not altered by the heat treatment. It may be concluded, therefore, that the heat treatment (83° to 85° C.) has resulted in fractionation of the proteins of the unheated globulin mixture by removal of those proteins which coagulate at temperatures up to 85° C.

Reactions of the uterine muscle from Animal 4026 (Fig. 1) were significant because they demonstrated again that muscles previously sensitized with purified preparations frequently become sensitive to some minor contaminating antigen which is incompletely removed by the process of purification.

TABLE VI. ANTIGENIC RELATIONSHIP OF CSG AND CSG-H

ANIMAL	SENSITIZING ANTIGEN	INCUBATION PERIOD (DAYS)	HORN NO.	PREPARATION ADDED	PROTEIN NITROGEN UNITS	UTERINE CONTRACTIONS
3681	CSG 1.0 mg. P.N. i.a.	24	1	CSG-H	10,000	++++
				CSG	10,000	++++
				Histamine		++++
			2	CSG	10,000	++++
				CSG-H	10,000	0
				Histamine		++++
3730	CSG 1.0 mg. P.N. i.a.	27	1	CSG-H	10,000	++++
				CSG	10,000	±
				UCS	10,000	0
				Histamine		++++
			2	CSG	10,000	++++
				CSG-H	10,000	0
				UCS	10,000	0
				Histamine		++++
4018	CSG 0.5 mg. P.N. i.a.	20	1	CSG-H	1,000	+++
				CSG	1,000	++++
			2	CSG	1,000	++++
				CSG-H	1,000	0
				Histamine		++++
3677	CSG-H 1.0 mg. P.N. i.a.	23	1	CSG	10,000	++++
				CSG-H	10,000	0
				Histamine		++++
			2	CSG-H	10,000	++++
				CSG	10,000	0
				Histamine		++++
3701	CSG-H 1.0 mg. P.N. i.a.	26	1	CSG	10,000	++++
				CSG-H	10,000	0
				UCS	10,000	0
				Histamine		++++
			2	CSG-H	10,000	++++
				CSG	10,000	0
				UCS	10,000	0
				Histamine		++++
4026 (See Fig. 1)	CSG-H 0.5 mg. P.N. i.a.	22	1	CSG	1,000	++++
				CSG-H	1,000	0
				UCS(S)	1,000	++++
				Histamine		++++
			2	CSG-H	1,000	+++
				CSG	1,000	±
				UCS(S)	1,000	++++
				Histamine		++++

DISCUSSION

The experiments reported here demonstrate the utility of the Schultz-Dale technique in studying the homogeneity of antigenic protein preparations. The results also emphasize the inadequacy of the usual precipitative methods for complete separation of proteins of such markedly different solubilities as cottonseed globulin and CS-1A. CS-1A is very soluble whereas cottonseed globulin is essentially insoluble in pure water. Nevertheless, cottonseed globulin preparations purified by ten extractions with distilled water and ten to fifteen subsequent precipitations in water were shown to contain CS-1A on the order of 0.2 to 0.3 per cent and, in addition, a trace of another unidentified water-soluble protein. The order of concentration of contaminating proteins observed here are of negligible importance in the usual study of protein chemistry, but because of the extreme sensitivity of the immunologic reactions these contaminants may assume marked importance in determinations of allergenic activity.

SUMMARY

1. Extensively purified cottonseed globulin preparations were shown, by gross anaphylaxis and by the Schultz-Dale method, to retain protein reacting like CS-1A. These preparations were estimated to contain CS-1A in the order of 0.2 to 0.3 per cent.

2. A significant fractionation of the globulin preparation was accomplished by heating the globulin fraction at 83° to 85 ° C. for one hour.

3. Both the heated and unheated globulin preparations contained a water-soluble antigen which was distinct from CS-1A.

4. The unidentified, water-soluble antigenic protein may be present in cottonseed globulin preparations in sufficient concentration to impart allergenic properties to them.

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METHOD FOR EXPERIMENTAL CONTROL OF PARTICLE SIZE OF THERAPEUTIC AEROSOLS*

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THE treatment of the respiratory tract by aerosols bids fair to become of increasing importance. Thus, penicillin has been shown to form stable mists with properties suitable for therapeutic purposes.†

In a previous communication[2] one of us has shown that lowering the vapor pressure of mist particles by means of suitable substances like glycerol, propylene glycol, sugar, or urea modifies the nature of the mist produced by commercial nebulizers. In this way, the introduction of epinephrine 1:100 into the lungs in the inhalant therapy of asthma was improved because the mists provided were more permanent.

With ordinary commercial nebulizers, however, the particle size is not standard and is therefore not suitable for experimental work in which large numbers of very small particles need be introduced into the respiratory tract. It is evident that some form of centrifugation could be used for the elimination of larger droplets so that the particles comprising the mist may be very small.

We have adapted a principle outlined in unpublished work by Regner and cited by Freundlich.[3] This principle leads to centrifugation of the heavier particles, and mists are readily obtained in which the particle size is 2 μ in diameter or less. No moving parts are required in the centrifugal device to be described. The centrifugal device consists essentially of a coil of tubing so wound that the centrifugal force developed incidental to the flow of the gas through the coil acts on the larger particles which condense on the coil and return to the reservoir. The smaller particles are blown out of the end of the centrifuge tube.

Theoretical.—The force, F, acting on a particle moving in a circular path in a current of air is given by the equation,

$$F = M \times \frac{V^2}{r}$$

where M is the mass of the particle, V is the velocity, and r is the radius of curvature of the path. If the particles take a circular path, therefore, with the velocity being held constant, the force acting on the particles is the greater, the smaller the radius. This notion is not at all intuitive but will be clarified by the following examples. If two men stand at different points on the radius of a merry-go-round, it is evident that the one standing further from the axis of rotation will tend to be thrown off more than the man nearest the axis. However, in this case the linear velocity of the two men is different. This case is *not* analogous to the centrifugal fractionator. If, however, another example is used, the way in which the centrifugal fractionator operates on

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†It is of historical interest that this program was initiated by, and is under the direction of, the Technical Division, Office of the Chief, Chemical Warfare Service. The support of the Macy Foundation and the active cooperation of Dr. Frank Fremont-Smith enabled the technical developments to be extended to physiological and medical aspects of the problem. (See reference 1.)

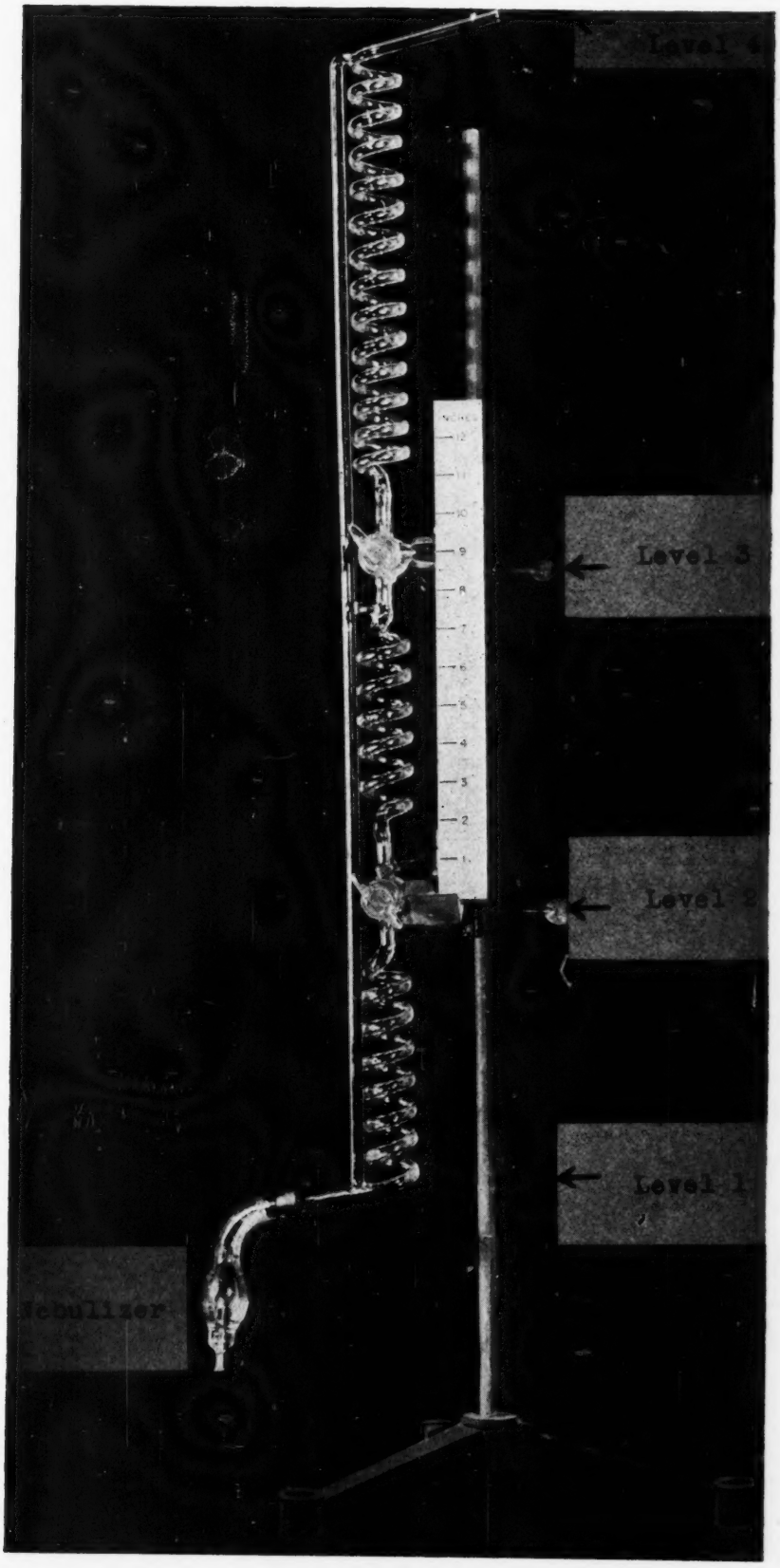

Fig. 1.

aerosols is clarified. A train going around a track at a given velocity, say, of 60 miles an hour, will not tend to be thrown off as much if the track has a great radius of curvature as if the track has a small radius of curvature. In other words, the particles in the centrifugal fractionator act in much the same way that a train does going around tracks of different radii of curvature with a constant linear velocity in each. Other factors, such as the distances separating the coils of the fractionator also play a role, but the treatment of these will not be considered at this point.

Experimental.—In these preliminary series of experiments, it was found that 50 per cent glycerol[2] formed stable mists with the DeVilbiss No. 40 nebulizer, the Parke-Davis nebulizer (table model) or the Vaponephrin nebulizer. As a matter of fact, the construction of the nebulizer is not as important for droplet size as the fractionator (itself) to be described. The fractionating column (Fig. 1) was a circularly wound tube 240 cm. long; the radius of the coils was about 1.5 centimeters. This was held vertically with the length of the column being broken up by two stopcocks, one at one-fourth of the level and one at one-half of the level. In this way samples could be obtained from: Level 1, from the nebulizer itself; Level 2, from the first stopcock one-fourth of the way; Level 3, one-half way; and Level 4, the entire length of the centrifugal fractionator.

Mists were obtained by applying 10 pounds of air pressure, and samples were collected in quart-size mason jars. These were inverted, and clean cover slips were quickly inserted to collect droplets for sampling. This method yields a preponderance of larger droplets but serves to illustrate the procedure. These samples were then studied with a magnification of 1,800 using a horizontal microscope arrangement projecting the image upward by reflection of a mirror. Drawings were made on tracing paper outlining the image obtained. The droplets obtained at different levels of the fractionating column may be divided into four groups for convenience: Class 1, diameter 8 μ to 38 μ; Class 2, diameter 6 μ to 8 μ; Class 3, diameter 2 μ to 6 μ; and Class 4, diameter 2 μ and below.

In obtaining the following data, cover slips were exposed to the mist from Level 1 directly from the nebulizer for thirty seconds; the others were exposed to samples from the mason jars, Level 2, the first stopcock, for sixty seconds; Level 3, the second stopcock, for ninety seconds; and from Level 4, the top of the column, for one hundred and twenty seconds. Calculations of droplet size were made by projecting the image of a micrometer scale through the microscope. Each division of this scale was 10 μ which was subdivided into ten parts.

Under the foregoing conditions, then, the following data were obtained:

	Size
Level 1 (from the nebulizer itself)	Class 4 – 7.2 per cent
	Class 3 – 65.5 per cent
	Class 2 – 18 per cent
	Class 1 – 9.4 per cent

In later runs for five seconds' exposure more of the smaller particles were observable. Many of the particles directly from the nebulizer are greater than 8 μ. This is readily observed on an inspection of a sample. At Level 2 most of the large particles had disappeared. A typical run is as follows:

Size

Class 4 – 86 per cent
Class 3 – 13 per cent
Class 2 – 1.1 per cent

At Level 3 the fractionation was even more spectacular. In a given run 96 per cent of the particles were in Class 4, whereas only 4 per cent were in Class 3. At Level 4, at the top of the fractionating column, all of the particles observed were less than 2μ in diameter, and, therefore, all fitted into Class 4.

It is of great interest that, with this simple type of fractionating column, quantitative control of aerosol particle size is readily provided.

DISCUSSION

It is of some interest that very few small particles, that is, less than 2 μ, are obervable when samples are taken directly from the nebulizer. If the exposure time is shorter, smaller particles are observed. The small particles are probably removed by collision with larger particles which grew at the expense of smaller ones. The fractionating column works in the following way. If the larger particles are propelled with high velocity against the side walls of the tube by centrifugal action, that decreases the probability of collision with the smaller particles which are thus liberated for transport along the tube for expulsion at the upper level.

SUMMARY

A simple centrifugal fractionator of aerosols for control of particle size is described.

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THE RESPONSE OF CONTACT DERMATITIS SITES IN ATOPIC INDIVIDUALS TO SUBSEQUENT STIMULATION WITH SPECIFIC WHEAL-INDUCING ATOPENS*†

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THE present study in atopic individuals was undertaken to determine the state of responsiveness of recently healed contact dermatitis sites to subsequent stimulation with specific wheal-inducing atopens. Bowman and Walzer,[1] in a previous investigation, studied the effects of wheals of both specific and nonspecific origin on the subsequent whealing power of the affected sites. Specific wheals were produced on the arms of pollen-sensitive subjects. One week later, the sites of these reactions were restimulated with the same atopen. The resulting wheals and erythemas were then compared with those elicited with the same stimuli at normal cutaneous sites on the same subjects. It was found that the wheals on the previously tested sites tended to be larger than those on the normal control sites. Likewise, when histamine instead of the atopen was used to restimulate the specific wheal sites, the resulting wheals were larger than those on the control sites. In another series of cases in which the primary wheal was produced by histamine, the sites showed no tendency to increased whealing reaction when they were subsequently restimulated. In all series, the erythematous reactions surrounding the wheals were weaker on the restimulated sites. In summary, the atopic wheal left the skin site more responsive to subsequent excitation by specific or nonspecific stimuli, whereas the histamine wheal did not.

The purpose of the present investigation was to determine what effect contact dermatitis, which is another type of specific inflammation of the skin, would have upon the subsequent whealing response of the affected tissues when a specific excitant was used for restimulation.

PROCEDURE AND TECHNIQUE

The subjects studied were adult patients with atopic hypersensitiveness who were known to be sensitive also to *Krameria* or poison ivy or both. Positive reactions of the contact type were elicited by means of the application of patch tests on the arms with a fluid extract of *Krameria*[2] or with a 5 or 10 per cent paste of poison ivy extract in lanolin. The sites of the reactions were marked with indelible pencil. They were not retested until evidences of the inflammatory process, such as redness, swelling, or desquamation, had disappeared. A faint pigmentation or glossiness of the skin, which occasionally persisted at the site of the contact reaction, was not considered a contraindication to the performance of the intracutaneous tests.

The sites of previous dermatitis were then tested by the intracutaneous method with atopens to which the subjects were known to be sensitive. Comparative tests were made according to the technique described by Bowman[3] in a previous communication. In each patient, a concentration of extract was used which preliminary testing had revealed would produce a wheal of slight (1 plus) or

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moderate (2 plus) degree. Reactions of this intensity had been found in previous studies to be more suitable for purposes of comparison than very marked or very slight reactions.[3] Control tests were done on normal skin at least 2 inches distant from the previously tested site. In one-half of the subjects tested, the control test was placed on the arm proximal to the contact dermatitis site and, in the other half, distal to it. This alternation of position was necessary in order to counteract the advantage in whealing response to a specific stimulus, which a proximal position on the arm possesses as compared to a more distal site.[3]

Readings were made when the intracutaneous reactions at the dermatitis site and its control had reached their heights. The size of the wheals and also the intensity of the edema at each wheal site were noted. The extent and intensity of the erythemas surrounding the wheals were also recorded.

Forty-seven sites of contact dermatitis were tested. Twenty had been the seats of reactions to *Krameria* and twenty to poison ivy. In seven instances, the same skin site had been the seat of two dermatitis reactions. In these seven cases, a second dermatitis reaction had been induced at the same site with the second excitant, after the reaction to the first excitant had completely subsided.

The intracutaneous tests were performed on forty previous dermatitis sites with an extract of combined giant and dwarf ragweed pollen. Timothy pollen extract was used on three sites, silkworm extract on three, and goat epithelium extract on one. The interval of time between the application to a skin site of the contactant causing the dermatitis and the intracutaneous injection of the wheal-inducing atopen was from 28 to 108 days; the average was 48.7 days. When the contact site had been stimulated by successive applications of *Krameria* and poison ivy extracts, the interval was computed from the time of application of the second contactant.

RESULTS

Comparative tests were performed on forty-seven subjects. The wheals induced with a specific excitant on the site of a previous contact dermatitis were larger than those produced with the same technique on the normal skin in 63.8 per cent of the cases (Table I). In 14.9 per cent of the cases, the wheals were equal in size. In 21.3 per cent, the wheals on the control sites were larger. These results semed to indicate that sites of contact dermatitis acquire an increase in susceptibility to excitation by specific wheal-producing allergens.

The findings were further analyzed to determine the effect of the intensity of the original dermatitis reaction on the degree of subsequent whealing. The original sites of dermatitis had been graded from 1 to 4 plus, denoting mild, moderate, marked, and spreading reactions. In comparing the degrees of wheal formation on the 1 plus sites with those on the control sites, only small differences in intensity were noted (Table I). Wheals on those dermatitis sites were larger than, and smaller than, the controls in 25.5 per cent and 19.1 per cent of the cases, respectively, while, in 8.5 per cent of the cases, both wheals were equal. However, the differences were appreciable when comparisons were made between the responses on the 2, 3, and 4 plus dermatitis sites and those on normal skin. The wheals produced at these areas exceeded the controls in 38.3 per cent of the tests, and were smaller in only 2.1 per cent. In 6.3 per cent of these cases, the wheals were equal in size.

As noted in earlier studies on re-excitation of sites of previous wheal reactions, results obtained in relation to erythema do not conform with those in

TABLE I. RELATION OF INTENSITY OF DERMATITIS REACTION TO SUBSEQUENT WHEAL AND ERYTHEMA PRODUCTION

INTENSITY OF REACTION OF DERMATITIS SITE	NUMBER OF SUBJECTS AND PERCENTAGE +	++	+++	++++	TOTALS
Wheal on test site larger than wheal on control site	12 (25.5%)	10 (21.3%)	6 (12.8%)	2 (4.2%)	30 (63.8%)
Wheal on test site equal to wheal on control site	4 (8.5%)	0	2 (4.2%)	1 (2.1%)	7 (14.9%)
Wheal on test site smaller than wheal on control site	9 (19.1%)	0	1 (2.1%)	0	10 (21.3%)
Erythema on test site larger than erythema on control site	4 (8.5%)	0	1 (2.1%)	0	5 (10.6%)
Erythema on test site equal to erythema on control site	8 (17%)	3 (6.4%)	0	0	11 (23.4%)
Erythema on test site smaller than erythema on control site	13 (27.7%)	7 (14.9%)	8 (17%)	3 (6.4%)	31 (66%)
Total number of sites	25	10	9	3	47

relation to wheal formation. The erythemas on the dermatitis sites were larger or more intense than those on the control sites in only 10.6 per cent of the cases. The erythema response was smaller on the dermatitis sites than on the controls, in 66 per cent of the cases, and equal on both sites in 23.4 per cent.

DISCUSSION

From the foregoing evidence, it is apparent that contact dermatitis alters the behavior of human skin in respect to subsequent whealing power. In the present study, this effect was determined at 28 to 108 days or at an average of 48.7 days, following the onset of the initial reaction. Furthermore, it is noteworthy, in comparing the results obtained in this study with those in the previous investigation by Bowman and Walzer,[1] that an immunologic reaction in the skin, whether of the contact (eczematous) type or of the whealing type, leaves the skin more responsive to subsequent stimulation with specific wheal-inducing excitants. This effect is the opposite to that found at the site of a wheal produced by a nonimmunologic excitant, such as histamine. The nature of the response of a contact dermatitis site to restimulation with a nonspecific excitant will be discussed in a future communication.

Both experiments demonstrated that the erythematous response at the restimulated sites was almost invariably less than that at the normal control sites. This finding supports the prevalent impression that the mechanisms responsible for the production of whealing and erythema are different.

TABLE II. EFFECT OF RELATIVE LOCATION OF TEST AND CONTROL SITES ON RESULTS OBTAINED

	TEST SITE PROXIMAL TO CONTROL	TEST SITE DISTAL TO CONTROL	TOTALS
	Twenty-four subjects	*Twenty-three subjects*	
Wheal on test site larger than wheal on control site	17 (70.8%)	13 (56.5%)	30 (63.8%)
Wheal on test site equal to wheal on control site	2 (8.3%)	5 (21.7%)	7 (14.9%)
Wheal on test site smaller than wheal on control site	5 (20.8%)	5 (21.7%)	10 (21.3%)
Erythema on test site larger than erythema on control site	2 (8.3%)	3 (13%)	5 (10.6%)
Erythema on test site equal to erythema on control site	4 (16.7%)	7 (30.4%)	11 (23.4%)
Erythema on test site smaller than erythema on control site	18 (75%)	13 (56.5%)	31 (66%)

A comparison of the results obtained with retests of sites which were respectively proximal and distal on the arm to the control site is afforded in Table II. The test site was proximal to the control in twenty-four of the forty-seven subjects studied. In this group, the wheals induced on the sites of previous contact dermatitis were larger than those produced on the normal skin in 70.8 per cent of the tests. In the group of twenty-three subjects in whom the test site was distal to the control, the wheals on the dermatitis sites were larger than those on the normal skin in 56.5 per cent of the cases. The average for both groups was 63.8 per cent. In the two groups the wheals on the test and normal skin sites were equal in 8.3 per cent and 21.7 per cent, respectively. They were smaller on the dermatitis sites in 20.8 per cent and 21.7 per cent, respectively. The advisability of reversing the relative positions of test and control sites in comparative studies is thereby demonstrated and the original findings of Bowman[3] are again confirmed.

SUMMARY

1. When an area of skin in an atopic individual is stimulated by contact with a specific excitant and becomes the site of a contact dermatitis, its response to specific wheal-inducing atopens becomes altered.

2. When the dermatitis reaction has completely subsided, an intracutaneous test at this site with a specific wheal-inducing atopen, such as pollen or animal dander, tends to induce a wheal formation which is larger than that obtained at a normal site on the same subject.

3. The more intense the original dermatitis reaction is, the more pronounced is the tendency to increased wheal formation upon subsequent specific excitation.

4. Erythema formation on restimulated sites tends to be less intense than on normal sites.

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THE NONANTIGENIC PROPERTY OF BEESWAX*

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SINCE the advent of penicillin and its use in the treatment of infectious diseases, the one disadvantage which has inconvenienced the patient as well as the nursing staff, has been the rapid excretion of penicillin through the kidneys, thus causing the frequent repetition of its injection. In 1943, Rammelkamp and Keefer[1] reported their observations on the excretion of penicillin. In the same year, 1943, Rammelkamp and Bradley[2] suggested diodrast as a substance which might decrease elimination. They administered 9,600 Florey units intravenously per hour to normal subjects and discovered that the excretion was retarded by the addition of diodrast to the infusion.

Delayed absorption has been successfully accomplished by two other methods, first, the method of prechilling for two hours the area into which the penicillin is to be injected, reported by Trumper and Hutter[3] in 1944; and second, the method of mixing penicillin with beeswax and peanut oil, reported by Romansky and Rittman.[4] The prechilling procedure is time-consuming but the latter method seems ideal if the agents with which penicillin is combined are harmless to the patient.

In the Johns Hopkins Hospital, many thousands of injections of epinephrine hydrochloride (adrenalin) in peanut oil as a vehicle and countless injections of bismuth in peanut oil have been administered intramuscularly and subcutaneously without the recording of a single reaction which could be credited to the oil. Reports have appeared in the literature questioning the safety of peanut oil, but, in my experience, the reactions which have occurred are due to chemically impure oil contaminated with the protein fraction of the peanut.

The antigenic property of beeswax thus far has not received any adequate study with regard to the possible allergic reactions which might occur after intramuscular or subcutaneous injection. Since beeswax may be considered the best agent so far developed to use with peanut oil as a means to slow penicillin absorption and its subsequent excretion, the studies of this paper were undertaken, and the conclusions are reported.

The physical character of beeswax has a direct bearing on the antigenic study. Beeswax melts at 67° C. (155° F.) and it is soluble in peanut oil in a concentration of 4 per cent by weight. This mixture remains in solution; there is no tendency for the wax to separate from the oil. The mixture even remains fluid at room temperature. A more concentrated mixture tends to become as thick as cold cream on cooling to room temperature and, when heated, it is still too heavy for intramuscular injection. Beeswax is less soluble in sesame oil and cottonseed oil than in peanut oil, and a 4 per cent mixture in these oils, unlike peanut oil, separates on standing (the wax settles to the bottom of the container).

The subcutaneous and intramuscular injection of beeswax in oil is painless, and absorption is perfect. This observation was reported by Cento,[5, 6] in 1937.

*The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

†From the Protein Clinic of the Medical Department of the Johns Hopkins Hospital.

He prepared a 5 per cent mixture of beeswax in olive oil and administered the material to patients suffering with tuberculosis. His injections were given in a series of five doses, an injection every second day; then, after a lapse of ten days, a second series of five doses was given, and so on after intervals of ten days until five such series had been completed. The total amount of wax per patient in this series was 7.5 grams. This was given intramuscularly without any reaction whatever in a large number of tuberculous patients. Cento's work has since been repeated by another Italian, Spaltro,[7] with no ill effects from the injections of wax and oil.

To prove whether or not beeswax has any antigenic properties, the following studies were made:

1. The microscopic examination of samples of unfiltered, crude, mixed beeswax for the presence of pollen grains.

2. The microscopic study of samples of filtered mixed beeswax.

3. The comparison of scratch tests, performed on known pollen-sensitive patients with 4 per cent crude and filtered beeswax mixed with peanut oil, and scratch tests of 4 per cent crude and filtered beeswax to which had been added:

   a. One cubic centimeter of pollen extract (30,000 units) to 9 c.c. of 4 per cent beeswax-peanut oil mixture, diluted 1:10 with peanut oil.
   b. One cubic centimeter of pollen extract (30,000 units) to 9 c.c. of 4 per cent beeswax-peanut oil mixture, diluted 1:100 with peanut oil.
   c. One hundred milligrams of dried pollen to 9.9 c.c. of 4 per cent beeswax-peanut oil mixture.

As beeswax might be contaminated with the pollen from trees, grasses, and common ragweed, individual mixtures were made of these three varieties, and tests were performed with the three pollen species in each of the cases in this study.

4. The intracutaneous tests with peanut oil, with 4 per cent crude unfiltered beeswax, and with 4 per cent filtered beeswax. These tests were performed on thirty-one patients, all of whom were sensitive to the pollen of ragweed, grass, and trees, or to combinations of these three varieties of pollen.

Problems 1 and 2.—Careful microscopic examination of thin heated layers of beeswax revealed no pollen of any variety in the unfiltered or filtered material.

Problem 3.—Scratch tests performed on twelve patients sensitive in varying degrees to the pollen of grasses showed no reaction whatever to the 4 per cent mixture of *unfiltered* crude beeswax. To the three control tests, however, there were reactions depending upon the sensitiveness of the individual subjects to the different pollen-beeswax-oil mixtures.

These same twelve patients were tested similarly with the same mixtures of *filtered* beeswax and pollen. The results of the tests were identical to the results obtained with the unfiltered material. It was necessary to use grass-pollen extract as well as dried grass-pollen grains in this experiment, as it is conceivable that in the formation of the wax, possibly contaminated by pollen, an extract might be made which could not be removed by the process of filtration. no reaction occurred, however, in any of these patients when tested with the filtered beeswax-oil mixture.

Because beeswax might be contaminated with all varieties of pollens, a series of tests were performed on patients sensitive to mixtures of tree pollens, to mixtures of grass and plantain pollens, and also to the pollen of ragweed.

TABLE I. REACTIONS FROM SCRATCH TESTS WITH CRUDE UNFILTERED BEESWAX IN PEANUT OIL AND WITH TWO DILUTIONS OF BEESWAX OIL TO WHICH HAD BEEN ADDED CONCENTRATED POLLEN*

NUMBER OF PATIENTS	UNFILTERED CRUDE BEESWAX (4 PER CENT) IN PEANUT OIL	CONTROLS: UNFILTERED CRUDE BEESWAX (4 PER CENT) IN PEANUT OIL TO WHICH HAS BEEN ADDED:		
		1 C.C. GRASS POLLEN EXTRACT (30,000 P.N.U.† PER C.C.) TO 9 C.C. WAX-OIL MIXTURE DILUTED 1:10 WITH PEANUT OIL	1 C.C. GRASS POLLEN EXTRACT (30,000 P.N.U.† PER C.C.) TO 9 C.C. WAX-OIL MIXTURE DILUTED 1:100 WITH PEANUT OIL	100 MG. OF DRIED GRASS-POLLEN GRAINS TO 9.9 C.C. WAX-OIL MIXTURE
12	All negative	6 Positive 6 Negative	6 Positive 6 Negative	11 Positive 1 Negative
		Ragweed Pollen Substituted for Grass Pollen		
12	All negative	8 Positive 4 Negative	8 Positive 4 Negative	11 Positive 1 Negative
		Tree Pollen Substituted for Grass Pollen		
4	All negative	4 Negative	4 Negative	3 Positive 1 Negative

*Filtered beeswax substituted for unfiltered wax gave identical results. A third control contained dry pollen grains mixed with wax and oil.

†Protein nitrogen units.

TABLE II. COMPARISON OF REACTIONS OF PEANUT OIL, CRUDE UNFILTERED BEESWAX AND FILTERED BEESWAX AS DETERMINED BY INTRACUTANEOUS TESTS*

NUMBER OF PATIENTS	1 PEANUT OIL	2 UNFILTERED CRUDE BEESWAX IN PEANUT OIL	3 FILTERED BEESWAX (4 PER CENT) IN PEANUT OIL
31	9 Entirely negative reactions	9 Entirely negative reactions	7 Entirely negative reactions
	22 Equivocal† reactions (Wheals averaged 0.5 cm.; redness, 1.5 cm.)	22 Equivocal reactions (Wheal averaged 0.5 cm.; redness, 2.0 cm.)	24 Equivocal reactions

*Amount of material injected, 0.025 c.c.

†An equivocal reaction in my opinion is of no significance, and can be considered of no immunological or clinical importance.

The results of the tests are presented in Tables I and II. In no instance was there the slightest reaction to beeswax-peanut oil mixtures, while the material to which pollen extract or dried pollen had been added produced characteristic mild to severe reactions depending upon the clinical sensitivity of the patients.

Problem 4.—The intracutaneous tests were performed on thirty-one patients, all of whom were allergic to one or more varieties of pollen. One-tenth cubic centimeter of peanut oil, 0.1 c.c. of 4 per cent unfiltered crude beeswax in oil, and the same concentration of filtered wax in oil were injected intracutaneously into the forearm of each subject in the experiment. In every instance, the reaction to *peanut oil* was comparable to the reactions which occurred at the sites of injection of unfiltered crude beeswax and the filtered beeswax. Four patients reported that the slight urticarial wheals persisted for twelve hours after the materials had been injected, but there was no reaction of consequence in any of the thirty-one patients. Ten control persons who were nonallergic, gave the same type reaction as the patients who were clinically sensitive to pollen.

SUMMARY

1. No pollen could be identified in crude or filtered beeswax by microscopic examination.

2. Beeswax cannot be kept in solution for intramuscular injection in a concentration greater than 4 per cent wax by weight in peanut oil. In this mixture, there is no tendency for the wax to separate from the oil.

3. No reaction occurred by scratch or intracutaneous tests with 4 per cent beeswax-peanut oil mixture in any of the patients who were tested, even though they had clinical hay fever or asthma due to one or more varieties of pollens. The prepared control solutions gave typical reactions.

4. Crude unfiltered beeswax and filtered beeswax gave no reactions in any of the patients who were clinically allergic and the results of the studies indicate that beeswax, combined with peanut oil because of its nonantigenic character, should be an ideal medium for delaying the absorption of penicillin when administered by subcutaneous or intramuscular methods.

I am indebted to E. R. Squibb and Sons who furnished the beeswax.

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ALLERGY TO CHEMICALS IN FLOUR

A Case of Dermatitis Due to Benzoic Acid

K. A. Baird, M.D., Saint John, N. B., Canada

SKIN irritations peculiar to bakers have been known and described for over a century. Many cases of this bakers' dermatitis (bakers' itch, bakers' psoriasis) occurring among those exposed to wheat flour are known to be due not to wheat protein but to simple chemicals which are added for the purposes of bleaching the flour and preventing mold, or to other substances used in baking, such as spices, fruit juices, sugar, and flavoring agents.[1, 2] Schweisheimer,[3] described "bakers' dermatitis" as due to various agents, including so-called flour improvers. The chief improvers blamed were persulfates of ammonium and potassium. These, apparently are not used in Canada, but the article mentions that an outbreak of dermatitis occurred among bakers in New South Wales within a few months after the introduction of these substances to the activators used in doughmaking. It continued while the persulfate was used.

The following case is that of a baker whose allergic contact dermatitis was elicited by external exposure to flour. In this case the eruption was not caused by wheat proteins but by infinitesimal traces of an incorporated simple chemical, namely, benzoyl peroxide, or rather its residue, benzoic acid.

It has proved possible for the patient to continue his occupation as long as he uses a flour which contains no benzoic acid residue.

CASE REPORT

H. M., a young adult who had spent a good many years as a baker and recently had operated his own home bakeshop, developed periods of asthmatic wheezing and an increasingly severe skin rash for about one year. He was given some intradermal skin tests and told that he was sensitive to wheat. He was also given some inoculations in an attempt to hyposensitize him, with possibly some relief of his asthmatic symptoms. The rash, however, became worse.

When first seen by the author on July 14, 1943, he had been in the hospital for two weeks. He thought the rash had become worse in spite of treatment. He had a very severe dermatitis on his arms, shoulders, face, neck, and on the front of his chest. This extended over the areas which would be exposed while he worked in the shop wearing only a light, open-necked shirt. He had not been troubled with asthma for several weeks. Acting on the impression (which could not be proved) that the rash was being maintained, in part, by a low grade staphylococcal infection, the patient was given a course of mixed sensitized vaccine in addition to protection of the severely irritated areas by soothing ointment under a dressing. Wheat was removed from his diet. Rapid improvement permitted him to return home in about ten days. He continued to improve until the skin became practically clear. He reopened his bakery and tried to avoid direct contact with flour, but, at various times during August and September when he was exposed to the flour dust, the dermatitis recurred. He thought the bread flour irritated more than cake and pastry flour, the gluten content of which is higher. He also noticed a little asthmatic wheezing after these exposures. The possibility that his sensitivity was due not to the wheat protein but to the improvers in the flour deserved some attention. He brought samples of cake, bread, and pastry flours of the type ordinally used in the business and which were said to contain "improvers." He also brought a sample of a flour which did not contain "improvers." Patch tests were done with these four varieties of flour by wetting a small quantity and holding it on the skin of the front of the chest with a pledget of absorbent cotton and a square of adhesive plaster. At the end of twenty-four hours the three improved flours had each produced an area of tiny papules which was slightly itchy and very much resembled the areas of skin where the rash began to develop when the patient was exposed to flour dust in his bakeshop. The "non-improved" flour did not produce any apparent reaction. The patient was advised to clear all traces of the "improved" flours out of his shop and lay in a stock of "nonimproved" flour.

At about this time a kind friend advised him so strongly to go elsewhere for treatment that he went to one of the larger cities on the continent where he was seen by a very good dermatologist who referred him for examination to an otolaryngologist and an allergist. The former felt he had chronic septic tonsillitis.

The allergist did tests for approximately sixty articles of food and contactants which seemed possible allergens. Many were done by both scratch and intradermal methods. The positive reactions (to intradermal) of any consequence were as follows:

Barley, 7 plus	Corn, 3 plus	Oats, 3½ plus
Wheat, 10 plus	Yeast, 8 plus	String beans, 4 plus
Chicken, 6 plus	Stock dust, 10 plus	Virginia tobacco, 4 plus
Short ragweed, 2 plus		Mixed grass, 6 plus

He suggested various measures for the avoidance of these substances and suggested that the patient might be helped by a course of histamine-azo-protein.

In the meantime the author had written the Department of Agriculture in Ottawa and received the following information:

> Most millers now bleach flour and add about five parts per million of potassium bromate. Bleaching is done by one or more of three agents:
>
> Nitrogen trichloride in proportion of about one or two grammes per barrel reacts with the flour to produce hydrochloric acid and ammonia. Nitrogen peroxide is usually produced by an electric spark

giving nitrite as an end product, but in so trifling an amount that it is difficult to be sure whether the nitrite found in the flour is a natural constituent or the result of the reaction. When benzoyl peroxide is used the residue from this material acting on the flour is benzoic acid, the quantity being about ten parts per million.

After returning home the patient decided to take the author's advice and change to nonimproved flours. Since doing this he has been getting along very well. He eats wheat bread. On some occasions he develops a slight papular rash on his arms or chest, but apart from making him slightly apprehensive lest the old severe dermatitis should return this does not affect him seriously. He is unable to correlate the appearance of this rash with any exceptional exposures to anything. He feels rather sure that handling or eating commercial jams and pickles is followed by some skin reaction. These often contain sodium benzoate as a preservative!

Patch tests done with potassium bromate and benzoic acid in water gave no significant reaction. A patch test of 6 per cent benzoic acid in liquid petrolatum was applied. A very definite papular rash appeared in about six hours. This increased and became rather annoying so that, at about the end of sixteen hours, the patient removed the patch. Some ten hours after that, the area of the patch could be clearly seen as a slightly inflammed rectangle with a goodly number of papules. A control test with liquid petrolatum alone was negative.

After considerable correspondence the following information was obtained from the Department of Agriculture and Department of Health and Welfare, at Ottawa.

Government regulations require that the residual benzoic acid in flour shall not exceed seventy-five (75) parts per million calculated as benzoic acid (C_6H_5COOH).

Of seven samples of bleached flour from six companies examined by the Division of Chemistry (Department of Agriculture) several years ago, five contained benzoyl peroxide. The amounts varied from traces to 45 p.p.m. expressed as benzoic acid.

When Novadelox (the bleaching agent containing benzoyl peroxide) was not used, no benzoic acid was found in the flour.

The Assistant Director of Research for the makers of Novadelox indicated he had never heard of a case where benzoic acid was said to cause bakers' dermatitis. He estimated that $\frac{3}{8}$ of an ounce of their product per barrel of flour would yield not more than 18 parts per million. This information was obtained through courtesy of the company whose three flours gave positive patch tests and seemed to be the cause of the trouble in the case under discussion. Correlating it with the results of the Department of Agriculture's figures we must conclude that, in some cases, as much as $\frac{11}{12}$ ounce per barrel must be used by some flour companies to give the 45 parts per million previously reported.

The makers of the flour which gave no positive skin test, and which the patient has been using for over a year without getting dermatitis, object greatly to the use of so-called improvers such as potassium bromate. Therefore, they do not use them but state that they subject their commercial flour to a *very light* treatment of "Novadel." They also indicate that the possible *trace* of benzoic acid which results would be further reduced because their flour remains in storage for a period before shipment.

It seems fair to conclude that what at first seemed an allergy to wheat, and later to potassium bromate, was really due to benzoic acid from the bleaching agent. The benzoic acid was present in such slight trace (if at all) in the second flour used that it was innocuous. The commercial jams and pickles, which the patient suspected, usually contain benzoate of soda.

During the month of February, 1945, this baker was unable to obtain the "unimproved" flour which he had been successfully using for over a year, so he bought a bag of the usual standard commercial "improved" flour. He very promptly developed several areas of dermatitis on both hands and a slight amount of papular rash across the base of his neck and on the front of his chest. The rash disappeared rapidly after he reverted to the nonimproved flour. This event seems to be an additional control test, corroborating the fact that he is sensitive to the one flour and not to the other.

SUMMARY

1. A baker, who developed dermatitis from "improved" flours which also contained traces of benzoic acid, and who showed positive intradermal skin tests to wheat, was able to work with "nonimproved" flour containing little if any benzoic acid.

2. Patch tests corroborated this difference in sensitivity to the two types of flour.

3. Patch tests did not show hypersensitivity to potassium bromate but did show contact type eczematous hypersensitivity to benzoic acid.

4. The conclusion that this is a case of allergic contact type dermatitis due to benzoic acid seems justified.

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Editorials

Classification of Asthma

A REFERENCE to the classification of asthma appears in the subject index of only one of four recent books on Allergy or Asthma. There is one paper in the recent literature. One author reviews the literature. The others discuss the problem under etiology, types, or differential diagnosis of asthma.

The following classification of asthma has been helpful in the management of a large series of cases. It takes into account etiology, symptoms, signs, and response to treatment. It is more practical than academic. It can be improved. It is not entirely original. The criteria for classification are stated dogmatically for brevity.

A. Atopic, with the following characteristics: positive family, food, and inhalant histories; other allergic manifestations; pallor of the nasal mucosa and often of the uvula; cough which is late and tends to herald the end of the attack; sputum which is glairy, mucoid, and frothy; positive cutaneous and intracutaneous reactions to foods and inhalants; diffuse wheezing; and good response to adrenalin. The allergist treats this group successfully.

B. Infectious, with the following characteristics: Symptoms begin in a patient previously well until he contracts an acute respiratory infection with fever, purulent discharge from the nose, and cough with or without purulent sputum. It is described as "flu" or "grippe" or a "deep cold." Asthma often begins weeks or months after the acute phase of the respiratory infection although cough and purulent anterior or posterior nasal discharge persist. Subjective unilateral preponderance of nasal stuffiness is the rule. Cough precedes and dominates the attacks. When cough is productive, purulent sputum is raised. The nasal mucosa is red and swollen. Mucopurulent strands bridge one or both nasal cavities. Pus is common in the middle meatus after shrinkage. The antra or frontals may transilluminate unequally. Red streaks of lateral pharyngitis indicate the affected side. The paucity of atopic leads in the history and skin reactions is striking. Troublesome exacerbations are accompanied by fever and leucocytosis. Penicillin or sulfonamides may abort acute exacerbations. Adrenalin alone is not very effective. Codeine with ephedrine or adrenalin affords more relief. There is the impression that emphysema, bronchiectasis, or both develop rather rapidly. Purulent infection of the sinuses is rarely absent. Prompt cure of sinus infection is urgent. The allergist has little to offer.

C. Mixed atopic and infectious asthma has definite evidences of both. It is important to determine, from the history, which came first. Infection complicating primary atopy is *perhaps* more amenable to atopic and conservative rhinological management than is atopy added to infection. This large group requires the combined services of the allergist and the rhinologist. The allergist should be his own diagnostic rhinologist. The problem is not how but when and what rhinological procedures to do.

D. Nonallergic, noninfectious. This group is subdivided into asthma due to: physical allergy, bronchial stenosis, psychosomatic references, acute left

ventricular failure (cardiac asthma), emphysema with wheezing, so-called "intrinsic asthma," and that due to the nasobronchial reflex from nasal foreign bodies and polyps. Few cases lack an element of atopy or infection. This group is suggested by: precipitation by heat, cold, effort, or anxiety; nasal polyps, obstruction, or foreign bodies; wheezing only on exertion; preponderance of unilateral signs or symptoms during or between acute paroxysms; localized inspiratory stridor which persists; dyspnea which is chiefly inspiratory; relief of nocturnal dyspnea by digitalization. Physical and psychosomatic asthma are amenable to therapy. The prognosis is poor in other kinds of asthma in this group. Response to treatment is usually transient. The allergist must be trained in internal medicine to deal fairly with this group.

Pure forms of *A*, *B*, and of the subdivisions of *D* are the exception when asthma is chronic and of long standing.

—*O. S., Jr.*

Under "Correspondence" another suggested classification appears.
—*Ed.*

Correspondence

The Editor, THE JOURNAL OF ALLERGY,
Dear Sir:

At the present time there is no accepted method of referring quickly and accurately to the severity of asthmatic symptoms. After thinking the matter over for some time, I have evolved a system for my own use which works satisfactorily, and it occurred to me that other allergists might also find it convenient.

The cardiologist classifies cardiac inadequacy in four degrees, the first being the least serious, the fourth the most serious. Pathologists grade the malignancy of neoplasms on a similar scale of one to four. For years the surgeon has spoken of burns of various degrees and everyone knows immediately what is meant without a long description detailing the extent of the damage.

Asthma readily lends itself to a similar classification, which has the advantage of saving much time in describing cases and results, in general, in a more accurate recording of diagnoses. It may be argued that, as the case progresses, it may improve or get worse and so the initial diagnosis will not hold. This is quite true in asthma just as it is in heart disease; the diagnosis then has to be changed, following the practice of the cardiologist.

The classification which I am using is as follows:

First Degree Asthma.—Mild asthma. Essentially no interference with normal activity. Readily controlled by simple symptomatic therapeutic measures.

Second Degree Asthma.—Moderately severe asthma. Interference with normal activities definite, but able to keep going at reduced level. Simple symptomatic therapeutic measures effective, but required in large doses.

Third Degree Asthma.—Severe asthma. Pronounced interference with normal activities. Controlled only by injection therapy.

Fourth Degree Asthma.—Very severe asthma. Status asthmaticus. Semi-invalid or invalid. Not adequately controlled by any symptomatic therapy.

If such a classification were generally recognized, it would be adequate for general discussion and would save much time in describing and talking about asthma. For purposes of recording a diagnosis, a secondary classification is needed. The seasonal or perennial and the continuous or intermittent nature of the symptoms should be noted. This can be done

simply by adding these words. Thus, "3° asthma, perennial, intermittent 2 weeks" would mean a case of severe asthma requiring frequent injection therapy for the control of symptoms, resulting in much loss of time to the patient, occurring about every two weeks throughout the year. Again, "4° asthma, seasonal, fall, continuous" indicates very severe asthma occurring only in the fall but continuing unabated during the season. The saving in time and space is obvious.

The classification is not a complete statement of any case and it is not intended that it should take the place of a careful history. However, were such a classification generally accepted, there would result a great simplification in conversation and literature on the subject of asthma, and at the same time a more accurate picture of a particular case would be conveyed. By adding the two or three descriptive words, office and hospital records can be kept and classified much more accurately and simply.

I shall appreciate receiving your reaction to this plan.

ARTHUR B. BERRESFORD

608 East State Street
Ithaca, New York
June 8, 1945

Book Reviews

Hayfever Plants: Their Appearance, Distribution, Time of Flowering, and Their Role in Hayfever, With Special Reference to North America. Roger P. Wodehouse, Ph.D., New York, 1945, G. E. Stechert & Co., pp. 245, illustrated.

The author's purpose is "to interpret the botanical facts of hay fever in terms of their clinical significance." The treatment is strictly technical only in the important matters of nomenclature and classification. The descriptions are semitechnical and are frequently elucidated by the author's original drawings. These include the flowers and other parts of some fifty wind-pollinated plants and an equal number of typical pollen grains. Two-thirds of the book is devoted to fundamental botanical considerations of pollen allergy and to the author's general conclusions regarding the role of particular plant families and species. In the final third these considerations and conclusions are applied locally. References and indexes are ample and convenient.

The presentation is well organized and concise. The author's wide botanical experience and his evidently thorough acquaintance with allergy problems and literature are the monograph's stamp of authority. His artistic genius contributes no small part to its distinction. As the first and only book-length discussion of the outdoor problems of inhalant allergy, this reference volume will be welcomed by all physicians and scientists who are in any way concerned with such problems.

O. C. DURHAM.

American Academy of Allergy

NEWS AND NOTES

The Executive Committee of the Academy met at Toledo on April 21 and 22, and the following excerpts are from the minutes of that meeting.

Constitution.—Under constitutional changes, it was pointed out that the present method of electing Members and Fellows is very complicated. It involves filling out several forms by the applicant; much correspondence between members of the Credentials Committee; the furnishing of numerous reprints by the applicant; and finally a long wait until the actual election by the Academy members assembled in annual meeting.

After discussion of the above factors, a motion was made by Dr. Cohen, seconded by Dr. Criep, that the Constitution Committee suggest the necessary changes to simplify the method of admission of Members and Fellows. The motion was carried.

Enlargement of Advisory Committee.—In order to bring about closer relations with the Certifying Board for Pediatrics, it was proposed to add Dr. Oscar Schloss to the Advisory Committee. Accordingly, a motion was made by Dr. Chobot, seconded by Dr. Criep, that Dr. Oscar Schloss be appointed to the Advisory Committee of The Academy of Allergy, subject to his being certified in Allergy. His term will begin with the next annual meeting. This delay was recommended to avoid the necessity of changing the Constitution. The motion was carried.

Annual Meeting.—Dr. Feinberg stated that there is a good possibility that the next annual meeting may be held in Chicago as originally scheduled. After discussion, the following motion was made by Dr. Criep, and seconded by Dr. Chobot: (1) The Program Committee should send out a call to the membership for papers to be read at the next annual meeting in December. Such papers should be available by September 1. (2) If it is evident within sixty days of the time for the meeting that the meeting cannot be held, then the papers are to be published in THE JOURNAL OF ALLERGY as program papers. The motion was carried.

Pollen Survey Committee.—Dr. Walzer, Chairman of the Pollen Survey Committee, who was unable to attend the Executive Committee meeting because of the grave illness in his family, reported by letter to Dr. Swineford as follows:

The committee is organizing the ground work for a rather ambitious program. The men who are to do the pollen counts in various parts of the country, are gradually being lined up. It is our hope to establish standard methods which will be generally adopted all over the country so that there will be some basis for comparison when our counts are eventually published.

I have been investigating the question of the collaboration of our committee with the Committee on Aerobiology of the National Research Council. I find that this committee has not met since Pearl Harbor and has adjourned for the duration. I am awaiting the return of the chairman of this committee who is out of the country and will attempt to establish cordial relations with the committee.

The members of the committee have agreed that it is important to build soundly and we are, for the present at least, primarily interested in lining up the proper man and adopting basic technics and standards.

Treasurer's Report.—Dr. Criep reported total deposits of Academy funds in the Pitt National Bank, Pittsburgh, from Dec. 27, 1944, to April 19, 1945, of $7,704.92. Expenses since Dec. 27, 1944, have been $4,574.32, leaving a balance on hand of $3,130.60. Dr. Criep further stated that there is about $600.00 outstanding for dues from all Fellows and Members. About one-half of the seventy-five men in Military Service have already paid their JOURNAL subscriptions of $5.00.

The treasurer then moved that a Budget Committee of three members be appointed, and that the books be audited each year by a certified public accountant. Dr. Chobot seconded the motion, which was put to a vote and passed.

President Swineford then read an excerpt of a letter from Dr. Cooke, requesting that funds derived from postgraduate courses given under the auspices of the Academy be ear-

marked for the use of the Education Committee, and kept as a separate account in the Academy treasury.

A motion was made by Dr. Cohen, seconded by Dr. Bullen, that all funds, secured through activities of the Education Committee, be placed in the general Academy treasury, and allocated by the Budget Committee to the Education Committee. This action is necessary to conform to the duties of a budget committee. The motion was carried.

Education Committee Report.—In the absence of Dr. Cooke, Chairman of the Education Committee, Dr. Swineford read the following report from Dr. Cooke:

With regard to under-graduate and graduate work in allergy, studies are being made by the various sub-committees. This is a long time study and progress is bound to be slow. Little can be done until we return to more normal times in medical education as a whole.

In the post-graduate field however, work can be done and as you are aware, a course was given under the auspices of the American College of Physicians at the Roosevelt Hospital last October, 1944. A second course was given in advanced studies in allergy at the Postgraduate Hospital under the auspices of the Columbia University and sponsored by the American Academy of Allergy. This was a one week course of morning lectures and afternoon colloquia, conducted at various hospitals in small groups of eight. I am enclosing a copy of the report made to me by Dr. Spain who directed these courses and as you will see, there is a balance to our credit of approximately $1,800. It is the feeling of this group here in New York that this money should be carried in a separate account in the treasury of the American Academy of Allergy for the Committee on Education for educational work. If this is satisfactory to the Executive Committee, a check will be sent to the treasurer just as soon as the details of the expense have been completed. As shown in Dr. Spain's report to me, a definite figure has not yet been obtained.

It seems to me that post-graduate courses of the type that have been given here, could and should be given elsewhere in the country. There is no reason why such a course could not be given in other teaching centers as Chicago, St. Louis, Pittsburgh and Los Angeles. It is up to the local men to arrange and develop these courses. The Committee on Education will be more than happy to help out on request. The committee can use some of the funds at its disposal to promote these courses, advise them if necessary and pay traveling expenses where needed but the money for such expenses should be returned to the fund out of the proceeds of the course. Proceeds of the courses should be added to the Educational Fund.

Following the reading of this report, there ensued considerable discussion relative to holding a postgraduate instructional course in the Midwest this fall. It was felt this should be held at some teaching center, such as St. Louis, Ann Arbor, or Chicago. Dr. Feinberg moved, and Dr. Criep seconded, that a committee be appointed to report to the Executive Committee in one month on plans for a two-day regional teaching meeting under the auspices of the Education Committee, to be held in the Midwest. The motion was carried.

Affiliate Members.—Dr. Cohen reported that there were some fifteen allergy organizations in the United States, other than the two national societies. Dr. Criep moved, and Dr. Cohen seconded, that the Constitution Committee investigate and report on the advisability of establishing affiliate members or work out a method by which regional societies and their members can become affiliated with the Academy. The motion was carried.

Primer on Allergy.—At the last meeting of the Executive Committee in New York, the Education Committee was instructed to secure from suitable Academy members, a series of special articles on Allergy to be published serially in *The Journal of the American Medical Association.* Eventually, this series was to be published in the form of a Primer on Allergy. The secretary was instructed to remind the Education Committee of this matter with a view to securing action.

New Committees.—The following new committees were appointed by the Chairman, Dr. Swineford:

Budget Committee

Dr. Leo H. Criep, Chairman — Dr. Robert A. Cooke — Dr. Karl D. Figley

Foundation Committee

Dr. Milton B. Cohen, Chairman	Dr. Stearns S. Bullen	Dr. Robert A. Cooke
Dr. Jerome Glaser	Dr. Karl D. Figley	Dr. Robert Chobot
Dr. Oscar Swineford, Jr.	Dr. Leo H. Criep	Dr. Samuel M. Feinberg
	Dr. Matthew Walzer	

Committee on Regional Meetings

Dr. Samuel M. Feinberg, Chairman — Dr. Milton B. Cohen
Dr. Leo H. Criep — Dr. Karl D. Figley

KARL D. FIGLEY, Secretary.

The School of Medicine, University of Pittsburgh and the American Academy of Allergy announce a postgraduate course in clinical allergy to be given at the School on Bayard Street, Pittsburgh, Pennsylvania, during the week of October 1 to 5, inclusive. This course will include lectures, demonstrations, case presentations, and laboratory procedures. The faculty is composed of distinguished allergists from various parts of the country.

The course will extend over a period of five full days. The fee is $40.00 and for men in Military Service, $10.00 Detailed information and applications may be obtained on request from William S. McElroy, M.D., Dean, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

The Academy acknowledges with thanks the receipt of a grant of $500 for the year 1945, from the Almay Company. This grant is for the purpose of furthering undergraduate education in the field of allergy.

The Committee on Pharmaceuticals and Medicaments urges that all requests from manufacturers of drugs and other antiallergic products sent to fellows or members individually for evaluation, be forwarded to the Committee. Under the supervision of the Committee such materials will receive thorough clinical investigation. The results of such study will enable the Committee to endorse valid claims and to reject those that are misleading. In the latter instance, it may have data to present to the Federal Food and Drug Administration. The Chairman of the Committee is Dr. Robert Chobot, 30 West 59th Street, New York City.

DIRECTORY OF THE AMERICAN ACADEMY OF ALLERGY

INTRODUCTION

WHEN the American Academy of Allergy was established in December, 1943, through the merger of the Society for the Study of Asthma and Allied Conditions and the American Association for the Study of Allergy, the Executive Committee of the Academy instructed the Secretary to prepare a Directory of Fellows and Members. In addition to the names and addresses, biographical data were to be included.

Many difficulties have been encountered in the preparation of such a directory due to the war, with the removal into active Military Service of many of our members. This has delayed the completion of the volume beyond my term of office as Secretary of the Academy.

Comprehensive information on many members in the Service has been obtained, but only the addresses of others were available. Wherever possible the permanent addresses have been given rather than the military addresses.

Doubtless there are many errors in the Directory which it will be possible to correct in subsequent editions. It will be appreciated if the membership will send any additional information and suggestions to the present Secretary of the Academy, Dr. Karl D. Figley, whose cooperation in compiling this Directory I gratefully acknowledge.

W. C. SPAIN, M.D.

April 4, 1945
New York City

ABBREVIATIONS

A.	American
A.A.A.	American Academy of Allergy
A.A.A.S.	American Association for the Advancement of Science
A.A. Immunol	Association of American Immunologists
A.C.A.	American College of Allergists
Acad.	Academy
A.A.P.	American Academy of Pediatricians
A.C.P.	American College of Physicians
A.C.S.	American College of Surgeons
A.E.F.	American Expeditionary Force
Allerg.	Allergists
Appr.	Apprentice
A.M.A.	American Medical Association
Assoc.	Associate
Assn.	Association
Asst.	Assistant
Att.	Attending
b	born
Bd.	Board
Biol.	Biology, Biologic
Bur.	Bureau
Cert.	Certified
Chem.	Chemical, Chemistry
Chm.	Chairman
Clin.	Clinic, Clinical
Coll.	College
Comm.	Committee
Comdr.	Commander
Consult.	Consultant
Dept.	Department
Dermat.	Dermatological, Dermatologist, Dermatology
Diag.	Diagnostic
Dir.	Director
Div.	Division
Exp.	Experimental
F.	Fellow
Hosp.	Hospital
Immunol.	Immunology
Inst.	Institute, Institution
Instr.	Instructor
Intern.	Internal
J.	Journal
Lab.	Laboratories, Laboratory
Lect.	Lecturer
M.	Member
Maj.	Major
Med.	Medicine, Medical
O.P.D.	Outpatient Department
Otolaryng.	Otolaryngologic, Otolaryngologist, Otolaryngology
Path.	Pathologic, Pathology
Ped.	Pediatrician, Pediatrics

Phys.	Physician
Physiol.	Physiologic, Physiology
Pres.	President
Prof.	Professor
Pub. Health	Public Health
Radiol.	Radiologist, Radiology
Rhin.	Rhinological, Rhinologist, Rhinology
Sch.	School
Sec.	Secretary
Tbc.	Tuberculosis
Ther.	Therapeutics, Therapist, Therapy
Treas.	Treasurer
U.	University
Urol.	Urological, Urologist, Urology
Vis.	Visiting

FELLOWS OF THE ACADEMY

1939 **Abramson, Harold A.**, Edgewood Arsenal, Md.
b 1899. A.B., Columbia 1920; M.D., 1923; Maj., M.C., U.S.A.; Assoc. Med. Allergy, Mt. Sinai Hosp., N.Y.; Asst. Prof. Physiol., Columbia U.; M. N.Y. Acad. Med.; Acad. Sciences; A. Soc. Research in Psychosomatics; A. Physiol. Soc.; Soc. Biol. Chem.; A. Chem. Soc.; A. Phys. Soc.; Soc. Invest. Dermat.

1942 **Albert, Murray M.**, 20 Plaza St., Brooklyn 17, N.Y.
b 1906. B.S., N.Y.U. 1928; M.D., Syracuse U. 1931; Chief Allergy, Greenpoint Hosp., Bklyn, N.Y.; Asst. Att. Allergy, Jewish Hosp. Bklyn.; A.M.A.

1923 **Alexander, Harry L.**, 4952 Maryland Ave., St. Louis 8, Mo.
b 1887. A.B., Williams 1910; M.D., Columbia U. 1914; Maj., M.C. (World War I); Cert. in Intern. Med. 1936 and in Allergy 1939; Assoc. Phys. Barnes Hosp., St. Louis; Consult. Phys. St. Louis Maternity and City Hosps.; Prof. Clin. Med. Washington U.; F.A.C.P.; M. Assn. A. Phys.; A. Soc. Clin. Invest.; Central Soc. for Clin. Research; Soc. Exp. Biol. and Med.; A.A.A.S.; Harvey Soc.; Sociedad Argentina para el Estudio de la Alergia (honorary member); Past Pres. Soc. Study Allergy and Allied Cond. and of A. Assn. Study Allergy; Advisory Bd. A. Allerg.; Ed., J. Allergy.

1941 **Arbesman, Carl E.**, 40 W. North St., Buffalo 2, N. Y.
b. 1911. B.A., U. Buffalo 1931; M.D., 1935; Clin. Asst. Med., Buffalo Gen. Hosp.; Asst. Chief Allergy Clin., Clin. Asst. Med., Children's Hosp.; Asst. Med. and Instr. in Bacteriol. and Immunol., U. Buffalo; A.M.A.; Sigma Xi; Buffalo Allergy Club.

1924 **Baldwin, Horace S.**, 136 E. 64th St., New York 21, N. Y.
b 1895. B.S., Wesleyan 1917; M.D., Cornell U. 1921; Hosp. Appr. 1st C. U.S.N. 1918; Cert. in Intern. Med. 1942 and in Allergy 1942; Asst. Att. Phys. and Dir. Allergy, N.Y. Hosp.; Clin. Consult. Med. and Allergy, North Country Community, Nassau, and Dobbs Ferry Hosps.; Asst. Prof. Clin. Med., Cornell U.; N.Y. Acad. Med; A.M.A.; Chm., Section Med., N. Y. Acad. Med. 1943-1944.

1924 **Baldwin, Louis C. B.**, Route 2, Box 395, Phoenix, Ariz.

1931 **Barnard, James H.**, 140 E. 54th St., New York 22, N. Y.
b 1900. B.S., Syracuse U. 1923; M.D., 1927; Comdr., M.C., U.S.N.R.; Hon. Att. Allerg., N.Y. Eye and Ear Inf.; Natl. Bd. Med. Examiners.

1937 **Barrett, I. W.**, 232 Le Flore Ave., Clarksdale, Miss.

1925 **Becke, W. G.**, 912 Beaumont Bldg., St. Louis, Mo. Att. Phys. Barnes and St. Louis City Hosps. Instr. Med. Washington U.

1924 **Bell, Samuel D.**, 131 E. 74th St., New York 21, N. Y.
b 1892. A.B., Princeton U. 1913; M.D., Columbia U. 1917; Childrens Med. Serv., Bellevue Hosp.; Clin. Prof., Dept. Ped., N.Y.U. Med. Sch.; A.M.A.

1925 **Bengtsson, Otelia J.**, 60 E. 58th St., New York 22, N. Y.
b 1897. A.B., Cornell U. 1919; M.D., 1922; A.M.A.

1923 **Benson, Robert Louis,** 406 Medical Dental Bldg., Portland 5, Ore.
b 1880. A.B., U. Mich. 1902; A.M., 1904; M.D., Rush Med. Coll. 1910; Cert. in Intern. Med. 1939 and in Allergy 1941; St. Vincent's and Providence Hosps.; Doernbecher Memorial Hosp. for Children; Asst. Clin. Prof. Med., U. Ore.; F.A.C.P.; M. A.M.A.; A.A.A.S.; A. Heart Assn.; Pres., A. Assn. Study Allergy, 1940-1941.

1941 **Bernstein, Theodore B.,** 12th Reg. Disp., Camp Farmin, Texas.
b 1903. B.S., Crane Junior Coll. 1922; M.D., U. Ill. 1926; Maj., M.C., U.S.A.; Instr. Med. (Allergy) Northwestern U. Med. Sch.; M., Chicago Soc. Allergy, Sec.-Treas., 1940-1942; A.M.A.

1939 **Bernton, Harry Saul,** 1925 Eye St., N. W., Washington 6, D. C.
b 1884. A.B., Harvard 1904; M.D. 1908; Cert. in Intern. Med. 1937 and in Allergy 1941; Allerg. Providence Hosp.; Clin. Specialist, Allergen Invest., Bur. of Agr. and Industr. Chemistry, Agr. Research Administ. U.S. Dept. Agr.; Prof. Hygiene, Dental and Med. Depts., Georgetown U.; F.A.C.P.; A.A.A.S.; M. Wash. Acad. Sc.; Internat. Assn. Med. Museums; A. Assn. Bacteriol. and Path.; A. Bacteriol. Soc.; Biol. Soc. Wash.; A.M.A.; South. Med. Assn.; Med. Soc. Dist. Columbia; Pres., A. Assn. Study Allergy 1927-1928.

1940 **Black, James Harvey,** 1405 Med. Arts Bldg., Dallas 5, Texas.
b 1884. Premed. Southwestern U.; M.D., Southern Meth. U. 1907; Cert. in Intern. Med. 1937 and in Allergy 1941; M. Staff: Baylor, Parkland, Methodist, Med. Arts, and Freeman Memorial Hosps.; Prof. Clin. Med. Southwestern Med. Sch.; A.M.A.; A. Pub. Health Assn.; F.A.Soc. Clin. Path., Pres., 1929-1930; A.C.P.; A.A.A.S.; Chm., Sect. Path. and Physiol., A.M.A. 1931-1932; Pres., A. Assn. Study Allergy, 1934-1935 and Sec. 1937-1943.

1942 **Blanton, Wyndham Bolling,** 828 W. Franklin St., Richmond 20, Va.
b 1890. B.A., Hampden-Sidney Coll. 1910; M.A., U. Va. 1912; M.D., Columbia U. 1916; Sr. Surg. U. S. Pub. Health Serv., Reserve; Cert. in Intern. Med. 1937 and in Allergy 1943; Phys. Hosp. Med. Coll. Va.; Prof. Clin. Med., Med. Coll. Va., and Chief, Immunol. Clin.; F.A.C.P.; A.C.A.; A.M.A.; M., Richmond Acad. Med., Pres., 1929; Med. Soc. Va.; Va. Acad. Science; Tri-State Med. Assn.; South. Med. Assn., Vice Pres., 1942; A. Assn. History Med.; A. Clin. and Climatol. Assn.

1938 **Blumstein, George I.,** 1614 Locust St. Philadelphia 3, Pa.
b 1904. M.D., Temple U. 1929; Mt. Sinai Hosp., Phila.; Instr., Med. Temple Med. Sch.; A.M.A.; Phila. Allergy Soc.; Phila. Co. Med. Soc.; Penn. State Med. Soc.

1928 **Brown, Grafton Tyler,** 1801 Eye St., N. W., Washington 6, D. C.
b 1893. B.S., Georgetown U. 1917; M.D., 1919; Cert. in Intern. Med. 1937 and in Allergy 1941; Allerg. Doctors Hosp.; Consult. Staff Georgetown Hosp.; Courtesy priv. Providence and Emergency Hosps.; Lect. Allergy Georgetown U. Med. Sch.; A.M.A.; F.A.C.P.; F.A.C.A.; South. Med. Assn.; A. Ther. Soc., Sec. and second and third Vice Pres.

1927 **Brunner, Matthew,** 892 Eastern Parkway, Brooklyn 13, N. Y.
b 1895. M.D., Bellevue 1919. Assoc. Att. in Allerg., Bklyn. Jewish Hosp.; Att. Allerg. Bklyn. Jewish San. and Hosp. Chr. Dis.; A.M.A.

1926 **Bullen, Stearns Samuel,** 26 South Goodman St., Rochester 7, N. Y.
b 1887. A.B., Cornell U. 1909; M.D., 1912; Cert. in Intern. Med. 1937 and in Allergy 1940; Asst. Phys. and Chief Adult Allergy Clin., Strong Memorial and Rochester Munic. Hosps.; Consult. Phys. Rochester Gen. Hosp.; Asst. Prof. Med., Sch. Med. and Dent., U. Rochester; A.M.A.; Executive Comm., Soc. Study Asthma and Allied Cond.; Pres. elect, A.A.A., 1945.

1938 **Burrage, Walter Swan,** 330 Dartmouth St., Boston 16, Mass.
b 1895. A.B., Harvard 1918; M.D., 1926; Comdr., M.C., U.S.N.R.; Cert. in Intern. Med. 1942 and in Allergy 1942; Asst. Phys. Mass. Gen. Hosp.; Phys. New Eng. Deaconess Hosp.; Allerg. Newton Hosp.; Asst. Med., courses for Grad. Harvard Med. Sch.; F.A.C.P.; A.M.A.; Soc. Invest. Dermat. Comm. on Standards Soc. Study Asthma and Allied Cond., 1942.

1939 **Cazort, Alan Garner,** 503 Donaghey Bldg., Little Rock, Ark.
b 1900. B.A., Hendrix Coll. 1922; M.D., U. Ark. 1928; U. Hosp.; St. Vincent's Infirmary; Baptist State Hosp.; Asst. Prof. Med., U. Ark. Sch. Med.; A.M.A., Ark. State; Pulaski Co. Med. Socs., Pres., 1943; South. Med.; S-W Allergy Forum.

1927 **Chobot, Robert,** 30 W. 59th St., New York 19, N. Y.
b 1901. A.B., Columbia U. 1921; M.D., 1924; Cert. in Intern. Med. 1937 and in

Allergy 1937; Chief Ped. Allergy Clin., Post Grad. Hosp.; Asst. Chief Allergy Clin., Roosevelt Hosp.; Asst. Clin. Prof., Ped., Columbia U.; F., N.Y. Acad. Med.; A.M.A.; Sec., N.Y. Assn. Allergy Clinics; Pres., A.A.A., 1944.

1924 **Clowes, G. H. A.** (Ph.D.), Eli Lilly & Co., Indianapolis, Ind.

1940 **Cohen, Armand E.**, 517 Brown Bldg. 321 W. Broadway, Louisville 2, Ky.
b 1900. B.S., Purdue; M.D., U. Louisville 1924; Norton Mem., Jewish Hosps.; Chief, Allergy Clin., Gen. Hosp.; A.M.A.; Ohio Valley Allergy Soc.; Midwest Allergy Forum.

1931 **Cohen, Milton B.**, 10616 Euclid Ave., Cleveland 6, Ohio.
b 1894. M.D., U. Louisville 1915; Lt., M.R.C., 1917-1919. Cert. in Intern. Med. 1937 and in Allergy; Vis. Allergy St. Alexis Hosp.; Dir., Asthma, Hay Fever and Allergy Fndn.; F.A.C.P.; A. Coll. Chest Phys.; M., A.A.Immunol.; Soc. Exp. Biol. and Med.; Executive Comm., A.A.A.; Past Pres., Soc. for Study of Asthma and Allied Cond.; Past Pres., A. Assn. Study Allergy.

1932 **Colmes, Abraham**, 416 Marlborough St., Boston 15, Mass.

1923 **Cooke, Robert Anderson**, 60 E. 58th St., New York 22, N. Y.
b 1880. B.A., Rutgers U. 1900; M.A., 1904; D.Sc., 1925 (honorary); M.D., Columbia U. 1904; Civ. Consult. to Sec. War, 1944; Cert. in Intern. Med. 1936 and in Allergy 1936; Att. Phys. Dir. Dept. Allergy, Roosevelt Hosp.; Med. Consult. Vassar Brother's Hosp., Poughkeepsie, N. Y., and Elizabeth A. Horton Hosp., Middletown, N. Y.; A.M.A.; F., N. Y. Acad. Med.; A.C.P.; M., Assn. A. Phys.; A.A.Immunol.; A.Soc. Clin. Invest.; A.A.A.S.; Cosmopolitan Med. Club; Harvey Soc.; Sociedad Argentina para el estudio de la Alergia (honorary member); a Founder and First Pres., Soc. Study Asthma and Allied Cond., 1923; a Founder and Chm., Assn. Allergy Clinics Greater N. Y., 1938; Governor, A. Coll. Phys. 1930-1934, Regent, 1934-1940; First Vice Pres., 1941; Chm. Advis. Bd. A. Allerg., 1940; Consult. to Comm. Med. Educ., N. Y. Acad. Med.; Consult. to Council on Pharm. and Chem., 1930; A.M.A.; M., Exec. Comm. A.A.A.; Med. Consult. in Allergy to Comm. on War Time Grad. Med. Meetings, 1943.

1929 **Credille, Barney Alexander**, 813 Genesee Bank Bldg., Flint 3, Mich.
b 1890. Premed. East Texas Normal Sch. 1911; M.D., Tulane U. 1918; Chief Allergy Serv., St. Joseph's Hosp.; Consult. Allergy, Hurley Hosp.; M., A.C.A.; Mich. Allergy Soc.; Sec., 1942.

1927 **Criep, Leo H.**, May Bldg., 5th and Liberty, Pittsburgh 22, Pa.
b 1896. B.S., U. Pittsburgh 1918; M.D., 1920; Cert. in Intern. Med. 1936 and in Allergy 1938; Chief, Med. Serv., Montefiore Hosp.; Sr. Staff Intern. Med., Presbyterian and Passavant Hosps.; Med. Staff Children's and Magee Hosps.; Consult. Allergy, U.S. Veteran's Admin.; Asst. Prof. Med. Lect. Immunol. U. Pittsburgh; A.M.A.; F., Biol. Research Soc.; Pres., Soc. for Study of Asthma and Allied Conditions, 1937.

1938 **Crump, Jean**, 1930 Chestnut St., Philadelphia 3, Pa.
b 1892. Premed. U. Pittsburgh; M.D., Woman's Med. Coll. Pa. 1923; Cert. in Ped. 1937; Chief Ped., Hosp. Woman's Med. Coll.; Chief Ped. St. Christopher's Hosp.; Assoc. Prof. Ped. Woman's Med. Coll.; Assoc. in Ped., U. Pa.; A.M.A.; Phila. Ped. Soc.; Phila. Allergy Soc.; A. Med. Women's Assn.; Pa. State Med. Assn.; Phila. Co. Med. Soc.

1927 **Cunningham, Thomas Donald**, 1612 Tremont St., Denver 2, Colo.
b 1889. B.S., Dartmouth Coll. 1913; M.D., Harvard 1918; Cert. in Intern. Med. 1937 and in Allergy 1937; Staff, St. Luke's, St. Joseph's, and Children's Hosps.; formerly Asst. Prof. Med., U. Colo. Med. Sch.; F.A.C.P.; A.M.A.; Central Soc. Clin. Research.

1926 **Davidson, Marion Tabb**, 2937 Southwood Road, Birmingham 9, Ala.
b 1887. A.B., Howard Coll. 1906; M.D., U. Cinc. 1911; Maj., M.C.; Cert. in Intern. Med. and Allergy; Birmingham Baptist and South Highland Inf.; Assoc. Med. U. Ala. (inactive); F.A.M.A.; South. Med. Assn., Chm. S. Allergy Section, 1935; F.A.C.A.

(To be continued.)